Experimental Nutrition and Cognition

Influence of dietary phospholipids and proteins on performance

Brenda Jorissen

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Promotores:
Prof. Dr. J. Jolles
Prof. Dr. W.J. Riedel

Co-Promotor:
Dr. J.A.J. Schmitt

Beoordelingscommissie:
Prof. Dr. A.T.M. Jansen (voorzitter)
Dr. E. Hogervorst (University of Oxford, UK)
Dr. A. Honig
Prof. Dr. G. Hornstra
Prof. Dr. B. Leonard (National University of Ireland, Galway)

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1001 films, op vergankelijk materiaal
opgeslagen, gerestaureerd en bewaard
gebleven is het geheugen
(André Delvaux, 1989)

Aan tante Trui

Datawyse

Contents

1

Cognition and Nutritional Interventions: Aims and Scientific Questions

Introduction

Many people living in western countries complain of poor memory and cognitive function. Recent findings from the Maastricht Aging Study MAAS (Jolles et al., 1995) show that at least 50% of subjects aged 50 years and older consider themselves forgetful, and that this percentage increases further with increasing age (Ponds et al., 1997). The prevalence of cognitive problems is even higher in individuals suffering from neurological or psychiatric disorders, notably neurodegenerative diseases (Andreasen et al., 1999; Mega, 2000; Stocchi and Brusa, 2000), traumatic brain injury (Klein et al., 1996; Matser et al., 1999), depression (Burt et al., 1995), or schizophrenia (Krabbendam, 2000; Krabbendam and Jolles, 2002). In addition to the conditions described as “normal” and often severe cognitive impairment, a borderline condition is characterized as ‘Mild Cognitive Impairment’ (Petersen et al., 1999; Visser, 2000). Another specific condition which is often accompanied by mild cognitive impairment is the Premenstrual Syndrome (Man et al., 1999). These conditions affect large number of people. The high prevalence of cognitive dysfunction in the general population emphasizes the need to search for effective medicinal or nutritional interventions to combat cognitive impairment, because coping with cognitive dysfunction is a burden, even in the absence of dementia.

In 1996, no medicinal drugs had been reliably and repeatedly demonstrated to be efficacious for the treatment of mild cognitive impairment (MCI) (Riedel and Jolles, 1996). However, pharmacological agents such as tacrine, a cholinesterase inhibitor (ChEI), and rivastigmine, another ChEI, can have beneficial effects on cognitive function in the treatment of Alzheimer’s Disease (AD) or related conditions (Knapp et al., 1994). In addition, donepezil, another ChEI with reported efficacy in the treatment of AD, was found to improve flight simulator performance in healthy pilots older than 50 (Yesavage et al., 2002). With respect to drug treatment in another condition in which ‘normal’ subjects are involved, i.e. women who are characterized by temporary changes in cognitive functioning, in particular phases in the menstrual cycle, there are also some interesting recent studies. Studies of women who experience severe premenstrual symptoms and temporary changes in cognitive function have shown fluoxetine, a selective serotonin reuptake inhibitor (Diegoli et al., 1998; Steiner et al., 1995), buspirone, a partial 5-HT_{1A} receptor antagonist (serotonin: 5-HydroxyTryptamine; 5-HT) (Landen et al., 2001), to have beneficial effects.

While several epidemiological studies have shown associations between nutritional status and mild cognitive impairment, the mechanisms underlying this association have proven difficult to analyze. Epidemiological studies can only establish associations, whereas proof of a causal association between nutritional intake and cognitive performance can only be obtained in experimental studies conducted according to a controlled study design. This thesis therefore aims to contribute to our knowledge of the mechanisms underlying the beneficial effects of nutritional treatment on cognitive performance. Two nutritional manipulations were investigated in controlled, double-blind studies involving two different groups of subjects with mild cognitive impairment, namely, elderly individuals suffering from age-associated memory impairment (AAMI) and women of reproductive age suffering from premenstrual symptoms, such as cognitive impairment and low mood. In the first instance, elderly individuals suffering from AAMI were given daily supplements of phosphatidylserine, and their memory and other cognitive functions were assessed. Earlier experiments in animals and humans had indicated that this nutritional supplement had promising effects on age-related cognitive decline. In the second instance, women suffering from premenstrual symptoms were given a meal

enriched with the tryptophan-rich protein α-lactalbumin, and memory and mood were evaluated. Supplementation with α-lactalbumin is expected to affect the availability of serotonin in the brain. The studies of this thesis tested the hypothesis that nutritional manipulations may be a good alternative to pharmacological agents for the treatment of less severe cognitive disorders. This is because nutritional manipulations can be expected to have fewer side effects. Moreover, pharmacological agents have not yet been shown to have really robust cognition-enhancing effects. The aims and hypotheses of these studies are outlined below.

Cognitive impairment and aging

Aging is accompanied by decline in the performance of a wide variety of different cognitive tasks (Jolles, 1986), such as memory (Zarit and Zarit, 1998), attentional functions, planning, complex decision-making, and speed of information processing (West, 1996). Age-related cognitive decline can be a result of several biological factors, such as decreased neurotransmitter action (Bartus et al., 1982), impaired neuronal membrane function (Sun and Sun, 1979), or shrinkage of certain brain areas, such as the thalamic volume (Van Der Werf et al., 2001). Psychosocial and environmental factors also appear to be of importance, such as exposure to bright light (Nathan et al., 1999), subjective evaluation of one's own memory performance (Ponds et al., 1997), and frequency of social interactions (Stevens et al., 1999). It even appears that engagement in mental and social activities protects against age-related cognitive decline (Bosma et al., 2003a; Bosma et al., 2003b). In this thesis, the focus will be on biological factors such as phospholipids and on the mechanisms underlying the influence of nutritional interventions on neurotransmitter actions.

Definitions of age-related cognitive decline

Through the years, the concept of 'age-related cognitive dysfunction' has evolved. Crook and coworkers first proposed the concept of age-associated memory impairment (AAMI) (Crook et al., 1986), a concept which proved to be influential in the 1990s. Several criteria have been proposed with the intention to differentiate between cognitively impaired subjects with and without preclinical dementia. For AAMI, which is the mildest of these criteria, the cognitive performance of elderly individuals is compared with that of young adults, whereas for other criteria, such as age-associated cognitive decline (AACD) (Rediess and Caine, 1996), the cognitive performance of elderly individuals is compared with that of their peers.
The AAMI criteria are intended to identify healthy elderly individuals who do not suffer from disease-related memory problems but who show age-associated memory impairment sufficient to be of subjective concern (Crook et al., 1986). Another relevant concept is Late Life Forgetfulness (Blackford and LaRue, 1989). AAMI (Crook et al., 1986) is an age-dependent condition with a high prevalence among the elderly. For example, a prevalence of AAMI of 3.6% in individuals aged 40 years and older, and 7.1% in individuals aged 65 years and older has been reported (Coria et al., 1993).
Others found the criteria for AAMI and Late-Life Forgetfulness to be unreliable and subsequently promoted criteria for MCI (Smith et al., 1996), defined as subclinical cognitive deficits due to incipient dementia in elderly individuals. Subjects who fulfill the MCI criteria are at increased risk (10–12%) of developing Alzheimer's disease (Petersen et al., 1999).
Age-Associated Cognitive Decline (AACD) (Levy, 1994) was a better predictor of later dementia, with a 28.6% conversion rate, than MCI, which had a conversion rate of 11.1% over a 3-year period (Ritchie et al., 2001). Elderly people suffering from AAMI perform on at least

one of three memory tests at least one standard deviation below the mean established for young adults, whereas elderly people suffering from AACD (Rediess and Caine, 1996) score at least one standard deviation below the mean for their own age group on memory tasks. MCI seems to be an unstable condition, whereas AACD appears to be more stable (Ritchie et al., 2001). While the term AACD nowadays is used more often than the term AAMI, more elderly people fulfill the criteria for AAMI since their performance is compared with that of young adults. AAMI is considered to reflect the relatively 'normal' age-related decline in cognitive function which is not characterized by disease.

Premenstrual symptoms and cognitive performance

More than 75% of women of reproductive age suffer from at least mild premenstrual symptoms (Ramcharan et al., 1992), but not all of them fulfill the criteria for Premenstrual Syndrome (PMS) (Mortola et al., 1990). Women may, for instance, suffer from somatic and affective symptoms, such as breast tenderness, headache, depression, irritability, or angry outburst in the period of 5 days before the menses, but not show identifiable dysfunction in social or economic performance. Premenstrual Dysphoric Disorder (PMDD) (APA, 1994), an extreme form of PMS, has a baseline prevalence of 5.8% and a cumulative lifetime prevalence of 7.4% (Wittchen et al., 2002). Some women also experience worsened concentration and cognitive function throughout the menstrual cycle (Sommer, 1992), although these results are controversial. Long-term memory performance is worse during menstruation than during the luteal phase of the menstrual cycle (Phillips and Sherwin, 1992), although a better performance on tests of articulatory and fine motor skills and a poorer performance on tests of spatial ability during the late follicular phase compared to the menstrual phase have also been reported (Hampson, 1990; Hampson, 1995). Moreover, several authors have failed to show any such effects in well-designed studies (Gordon and Lee, 1993; Nakatani et al., 1993).
These cyclic fluctuations in the performance of cognitive tasks by women suffering from premenstrual symptoms could be used to model mood and/or cognitive changes associated with low mood or depression in general. This model could be used to test putative cognition-enhancing nutritional or pharmacological manipulations, by comparing the effects of such manipulations on performance during the premenstrual and postmenstrual phases of the menstrual cycle.

Nutrition and Cognition

Pharmacological or nutritional agents may influence cognitive performance at several levels in the central nervous system, for example, by influencing the release of different neurotransmitters, the permeability of the blood-brain barrier, signal transduction within neurons, or the availability of precursors of neurotransmitters (e.g. serotonin). From the few epidemiological studies and controlled experiments of the influence of nutrients on cognitive performance reported so far, it appears that several macro- and micronutrients, such as caffeine, carbohydrates (glucose), amino acids (tryptophan, tyrosine), vitamins and minerals, may enhance cognition (Bellisle et al., 1998; Dye et al., 2000; Jorissen and Riedel, 2002; Riedel and Jorissen, 1998). Thus, nutrition may have an important role in the treatment of premenstrual and age-related mild cognitive impairment, although claims need to be sub-

stantiated in carefully designed research. Many questions remain to be answered: Is it possible to delay cognitive aging with pharmacological or nutritional treatment? Can medication or certain types of food or food ingredients improve cognitive functioning in women suffering from cycle-dependent changes in mood and/or cognitive dysfunction?

Outline and aims of this thesis

The central question of this thesis is: Is it possible to improve cognitive performance in subjects with mild cognitive impairment by means of nutritional interventions? The first part (chapters 2 – 5) focuses on age-related cognitive decline, whereas the second part (chapters 6 - 7) focuses on serotonergic mechanisms of memory impairment in women with premenstrual symptoms. These experiments reflect a combination of the serotonergic research (Markus 1999) with two other approaches: menstrual cycle and appetite control research (Blundell, 1977; Dye and Blundell, 1997). The chapters of this thesis cover the following topics:

Chapter 2 and 3: Nutrients, Age, and Cognitive Function
This section provides a review of the recent literature regarding the influence of nutritional supplements on cognitive functioning in elderly individuals, with emphasis on the following questions.
Is age-associated cognitive decline an opportunity for nutritional intervention?
Is nutritional treatment a good alternative to pharmacological treatment?
Are there studies of good quality which deal with the subject ‘Nutrition and Cognition’?
Are there nutritional supplements which improve cognitive functions?
Is there a positive association between nutrients and cognitive performance in elderly individuals, and if so are there experimental models which can be used to confirm this association?
Information was retrieved from the literature using the Medline and Psychlit databases. Chapter 2 covers the literature published from 1990 to 1998, and Chapter 3 the literature published from 1998 to 2001. There are not many published experimental studies, and most of the reviewed studies were epidemiological, describing correlations between certain nutrients and cognitive performance.

Chapter 4: The Influence of Soy-derived Phosphatidylserine on Cognitive Functions in Age-Associated Memory Impairment (AAMI)
A double-blind, placebo-controlled clinical trial investigating the influence of two dosages (300 mg or 600 mg daily) of soybean-derived phosphatidylserine (S-PS) in 120 elderly individuals with age-related cognitive decline was carried out to answer the following questions.
Does S-PS improve cognitive functions? In particular, do elderly individuals with mild cognitive impairment treated with doses of 300 and 600 mg S-PS daily perform better on a test of delayed recall memory and various other tests of memory and attention at the end of a 12-week treatment period?
Is the possible treatment response associated positively with the severity of cognitive impairment at baseline? To this end, subjects were divided into 2 subgroups, mild and moderate, which was based on their cognitive performance at baseline. The subjects included in the mild group met the criteria for AAMI (Crook et al., 1986), while subjects included in the moderate group also met the criteria for AACD (Rediess and Caine, 1996).

Chapter 5: The Safety of Soy-derived Phosphatidylserine in Elderly People
The safety of bovine cortex phosphatidylserine (BC-PS) has been investigated earlier; however, since S-PS has largely replaced BC-PS, it is important to answer the following question:
Is S-PS a safe nutritional supplement according to the same standards applied to medicinal drugs, including standard biochemical safety parameters, hematological safety parameters, blood pressure and heart rate?

Chapter 6: Dietary Alpha-lactalbumin Increases the Ratio of Plasma Tryptophan to the other Large Neutral Amino Acids and Ameliorates Premenstrual Memory Consolidation Deficit
Serotonin concentrations fluctuate in a cyclic fashion during the menstrual cycle, and thus women who experience premenstrual symptoms in the premenstrual phase may be vulnerable to a nutritional manipulation aimed at increasing brain serotonin levels during the premenstrual phase when serotonin concentrations are at their lowest. In a double-blind, placebo-controlled, cross-over study, the cognitive effects of a standard diet enriched with a high concentration of a tryptophan-containing protein (α-lactalbumin) were evaluated to answer the following questions:
Are there cognitive differences throughout the menstrual cycle in women suffering from premenstrual symptoms? In particular, do women have an impaired performance on tests of memory and planning during the premenstrual phase as compared with their performance on the same tests in the post-menstrual phase of the cycle.
If so, can α-lactalbumin reverse this difference? In particular, do women perform tests of memory and planning in the premenstrual phase better when treated with α-lactalbumin than when treated with placebo?

Chapter 7: The effect of Alpha-lactalbumin on Mood and Appetite in Women Suffering from Prementrual Symptoms
Mood and appetite vary throughout the menstrual cycle (Steiner, 1992), and both have been related to serotonin levels (Blundell, 1992; Young and Goodwin, 1991). In conjunction with the previous chapter, the following questions are addressed:
Do mood and appetite differ throughout the menstrual cycle in women who suffer from premenstrual symptoms? In particular, are scores on scales measuring mood and appetite (in the direction of lowered mood, increased appetite, and increased carbohydrate intake) different in the premenstrual and postmenstrual phases of the cycle.
If so, can α-lactalbumin reverse these changes? In particular, are the scores for mood and appetite in the premenstrual phase of the cycle normalized after women are treated with α-lactablumin compared with placebo?

Chapter 8: Concluding Remarks
Chapter 8 concludes this thesis with a general discussion and concluding remarks concerning the central question, whether nutritional intervention influences cognitive performance.

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Nutrients, Age and Cognitive Function: Part I

1990 - 1998

Many nutrients or indices of nutritional status are associated with cognitive functioning, although the size of the effects on cognitive performance may be small. Results from recent studies, however, seem consistently to indicate that supplementation with β-carotene and α-tocopherol, substances that promote antioxidant vitamins A and E, respectively, can be beneficial to cognitive function in elderly people. Folate rather than vitamin B12 appears to be associated with cognitive functioning. Furthermore the daily intake of ginkgo biloba extract can enhance cognitive performance and has been proved to delay cognitive decline in dementia. A proper dietary composition with regard to the ratio of carbohydrates to proteins, as well as the inclusion of sufficient micronutrients, seems to be favourable in the maintenance of cognitive function in the elderly. Glucose can enhance cognitive function, but a rapid decline of glucose levels may impair cognitive function or may induce feelings of lack of energy. Low doses of caffeine may also enhance cognitive function, although most studies on caffeine and cognition, as with studies on glucose and cognition, have not been carried out in elderly individuals. The effects of nutritional supplements are modest but do not seem to be very different from those of medicinal or investigational cognition-enhancing or anti-dementia drugs.

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Introduction

Although traditionally it has been assumed that the vast majority of those living in industrialized countries have an adequate micro-nutrient intake, there is a growing interest in the suggestion that supplementary intake may have advantages for at least some in the population (Benton et al., 1995). It has been argued that the first symptoms associated with micronutrient deficiency are psychological (Benton, 1992). Despite the widespread concern over the increasing problem of age-associated cognitive decline, little research has been devoted empirically to studying the influence of nutritional supplements in human cognitive functioning, particularly in the elderly.

The borderland between normal and pathological ageing

Cognitive ageing, or age-associated cognitive decline, is a phenomenon that is characterized by the decline of many aspects of cognitive functioning with age (Birren and Schaie, 1990; Charness, 1985; La Rue, 1992). There is a borderline between so-called ‘normal cognitive ageing’ and pathological conditions such as dementia, notably Alzheimer’s disease (AD), in which there are disorders of memory and other cognitive functions (Masur et al., 1994). Dementia and related pathological conditions, including prodromes of dementia, have a great impact on society because of the financial and organizational consequences for both families and society (Jolles et al., 1995). Furthermore, the ageing of the population results in an increasing prevalence of age-associated cognitive decline, and a widespread request for therapeutic agents aimed at least at improving the conditions of cognitive impairment or halting cognitive decline (Pepeu, 1994).

Age-associated cognitive decline: an opportunity for intervention?

Although great progress has been made in our understanding of brain changes in elderly individuals with cognitive deficits or AD patients, a breakthrough in the treatment of these conditions does not seem to be at hand. Yet, there has been a rapid increase of interest in the development of substances (drugs or nutritional supplements) for palliative treatment of age-related cognitive deficits and AD conditions (Ban, 1994; Michel et al., 1994; O'Brien and Levy, 1992). As a consequence, many pharmacological compounds have been tested with regard to their effects upon memory, in animals, in healthy elderly humans, and in AD patients (Riedel and Jolles, 1996). Industries developing cognition-enhancing drugs have faced the problem that age-associated cognitive decline was, and is, not recognized as a disease. Among clinicians no consensus exists as to whether age-associated cognitive decline should actually be a therapeutic target for medicinal drugs (Porsolt, 1990). For this reason, nutritional supplements could be of more interest in this condition.

The borderland between nutritional supplements and medicinal drugs

The question as to the difference between nutrients and drugs is an important one, in the light of the previous paragraph. Rather than precisely describing this difference, however, we would like to illustrate the matter referring to the UK’s policy on vitamin B6 (Bender, 1998), wherein low daily amounts are considered to be a nutritional supplement and high daily amounts are considered to be a medicinal drug, which is available on prescription only. Two

issues will always underlie such policies: (1) the emergence of side-effects being the most important; and (2) the mechanism of action being pharmacological or non-pharmacological. Although the decision on vitamin B6 has been heavily criticized (Downing, 1998), it is an example of the thesis that there is also a borderland between nutritional supplements and medicinal drugs. The borderline can be demarcated, however, by political decision. The consequence of that decision is that the consumer has some reassurance, in the case of using the compound for treating a specific condition, that some cost-benefit risk has been considered by the health authorities. In the case of prescription, some evidence of efficacy and safety has to be given. Until now, in the claims of nutritional supplements that claim to enhance cognitive function, there has not been a strong tradition of proving efficacy in vivo, as witnessed by the scarce number of studies that could be found for making up this review.

Assessments of cognitive function

The central theme of cognitive psychology is that of the organisms' internal representation of the outside world (Hunt, 1993). For example, novice and experienced clinicians differ in the way they perceive their patients and subsequently form their diagnosis. The explanation is not that their perception is different, but that their internal representation of the problem, to which their perception is compared, differs. An internal representation thus cannot do without memory. Perceived images are stored in working- or short-term memory and compared with knowledge retrieved from long-term memory. Although memory is known as a function of the brain, it is certainly not one observable organ, but its vital functions, such as storage, search, consolidation and retrieval, can be assessed using neuropsychological tests, electroencephalographic (EEG) measures, clinical ratings, or elaborate computerized test batteries of cognitive function.

Most assessments of cognitive function include immediate and long-term verbal and visual memory, as assessed through free recall and recognition paradigms, although there are many batteries comprising different tests of cognitive functions. These include the speed and accuracy of visual and auditory perception, thinking and movement times in choice reaction and problem solving, shifting and focusing of attention, inhibition of dominant motor responses, sentence verification, lexical decision making and visuomotor praxis, etc. Physiological paradigms to assess cognitive function include the measurement of the amplitude and latency of the positive wave around 300 ms (P300) after the presentation of a stimulus in the averaged event-related potential derived from EEG recordings. Another physiological paradigm is the assessment of alertness from the measurement of spontaneous EEG. The mini-mental state evaluation (MMSE) (Folstein et al., 1975), the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog) (Rosen et al., 1984), the cognitive scale of the Cambridge mental disorder examination in the elderly (Roth et al., 1986) and the Hodkinson Abbreviated Mental Test (Cattin et al., 1997) can be considered clinically validated tests for assessing cognitive function in moderate to severe age-associated cognitive decline and dementia.

Vitamins

Ageing processes, and among them brain ageing, are thought to be associated with free radical action. It is hypothesized that plasma antioxidant vitamin levels correlate with cogni-

tive performance in healthy older individuals. Among the vitamins that may protect against oxidative damage are the A, B, C and E vitamins. Most previous studies into vitamins and cognitive function pertained to the B vitamins, B1, B3, B6 and B12 in particular. The association of vitamin B1 deficiency with memory dysfunction and cognitive disorders has previously been related to an impairment of cholinergic activity (Micheau et al., 1985). Vitamin B1 (sulbutiamine) has been advocated for the treatment of cognitive dysfunction and fatigue of central origin (asthenia), prevalent after prolonged physical exercise in endurance athletes (Consolis and Mas, 1988), but also in ageing (Israel et al., 1989). It has been suggested that the cognition-enhancing potential of thiamine in AD is equivalent to that of cholinesterase inhibition and due to its general lack of adverse effects would deserve the benefit of the doubt (Meador et al., 1993). Recently, the administration of vitamin B1 improved mood and reaction times, but not memory performance (Benton et al., 1997). After 2 years' treatment of dementia patients with α-tocopherol (vitamin E), delayed institutionalization was shown, manifesting delayed progression of the disease, presumably induced by the improved vitamin E status (Sano et al., 1997). In a study which was based on the idea of combating seasonal affective disorder (Lansdowne and Provost, 1998), vitamin D3 significantly enhanced mood for 5 days during late winter. There have been three relatively large correlational studies in which the association between vitamin status and cognitive performance in elderly people was studied (Hassing et al., 1998; La Rue et al., 1997; Perrig et al., 1997):

1. Among people aged 65 and older, a higher ascorbic acid and β-carotene plasma level was found to be associated with better memory performance (Perrig et al., 1997).
2. Various indices of improved cognitive performance in 137 elderly community residents were associated with higher current dietary intake of thiamine, riboflavin, niacin, folate plasma ascorbate, also with a higher past intake of vitamins E, A, B6 and B12 and finally also with the use of self selected vitamin supplements (La Rue et al., 1997).
3. Individuals with low folic acid levels, but not those with low vitamin B12 levels showed impairment in memory recall (Hassing et al., 1998).

Summarizing, the latest research on vitamins and cognition seem to confirm the known positive associations of vitamins A, B1, B2, B3, B6, C and E with cognitive function, whereas new findings are the possible mood-enhancing effects of vitamin D3 and the observation that folic acid may be more critical than B12 to memory functioning in later life, as it may exert an influence on brain protein synthesis.

Flavonoids, terpenoids: ginkgo biloba

Currently, herbal compounds known by ancient medicine in the far east are of growing interest in the domain of cognitive enhancers. One such compound, which is very popular, is ginkgo biloba extract (GbE). It is available over the counter as a nutritional supplement in many countries and as a medicine on prescription in a few. It is a typical example of a substance in the borderland between nutritional supplements and cognition and medicinal antidementia drugs. Its main indication is that of 'cerebral insufficiency', a condition comparable to age-associated cognitive decline. GbE has recently been approved in Germany for the treatment of dementia. The mechanism of action of GbE in the central nervous system is only partly understood, but the main effects seem to be related to its antioxidant properties, which require the synergistic action of the flavonoids, the terpenoids (ginkgolides, bilobalide),

and the organic acids that are the principal constituents of GbE (Le Bars et al., 1997). In 1992, a review of 40 clinical trials investigating the effect of GbE was published (Kleijnen and Knipschild, 1992). Only eight were judged to be of good quality. The latter revealed mainly positive effects of GbE. Recently, two controlled clinical trials were carried out with GbE (Kleijnen and Knipschild, 1992; Le Bars et al., 1997). GbE showed no side effects and was significantly superior to placebo in terms of stabilizing and, in a substantial number of cases, improving cognitive performance and social functioning in 200 demented patients for 6 months to 1 year. Although modest, the changes induced by GbE were objectively measured by the ADAS-cog and were of sufficient magnitude to be recognized by the caregivers using the Geriatric Evaluation by Relative's Rating Instrument (Le Bars et al., 1997). In our opinion this is the best described study that was published in the reviewed period. In another study (Wesnes et al., 1997), the cognitive effects of 90 days' treatment with placebo and different dosages of a combination of GbE with Panax ginseng were studied in 64 middle-aged individuals with neurasthenic complaints. The results showed that the treatment improved cognitive performance in a dose-related manner when measured 1 h after the morning dose, but surprisingly this was the first study ever published on GbE to mention its impairing effects, as impaired cognitive performance was observed in a dose-related manner, 1 h after the afternoon dose. As the treatment was given in two daily doses of 60 mg only 5 h apart, rather than the usual 40 mg three times a day regimen, the authors speculated that a short-term accumulation might explain these observations, suggesting that a longer inter-dosing interval would be preferable.

Manipulation of dietary intake and the association between nutritional status and cognition

Food deprivation of varying degrees has been found to affect cognition. Although it has previously been demonstrated that missing breakfast usually impairs cognitive performance because of a decreased availability of glucose in the brain (Benton and Sargent, 1992), it has also been found that brief fasting improves cognitive performance or has no effect on cognition even after 24 h of fasting (Green et al., 1995; Green et al., 1997). Longer periods of fasting lead to slower simple reaction times but other cognitive functions remain unaffected whereas word recall performance has been reported to improve significantly (Kretsch et al., 1997). A rise in epinephrine levels and a resulting rise in the brain blood glucose level may be the intermediary process in these cases. Three experiments exploring the role of breakfast and increasing blood glucose in improving memory function showed that breakfast consumption preferentially influences tasks requiring aspects of memory and that a glucose drink could reverse the performance decline induced by not eating breakfast on some but not all memory tasks (Benton and Parker, 1998). As well as the vitamin study by La Rue et al. (La Rue et al., 1997), in which improved cognitive performance was also associated with higher current dietary intake of protein, and with serum albumin or transferrin, there were three other correlational studies in which the association between nutritional status and cognitive performance in elderly people was explored (Cattin et al., 1997; Kalmijn et al., 1997; Ortega et al., 1997):

1. In 260 healthy elderly people, individuals with adequate cognitive function, as assessed by the relatively crude MMSE, had lower intakes of monounsaturated fatty acids, saturated

fatty acids, and cholesterol, and higher intakes of total food, vegetables, fruit, carbohydrate, fibre, thiamine, folate and vitamin C, β-carotene, iron and zinc (Ortega et al., 1997).
2. High linoleic acid intake was associated with cognitive impairment after adjustment for age, education, cigarette smoking, alcohol consumption, and energy intake in 390 elderly men. Intake of n-3 polyunsaturated fatty acids was not associated with cognitive impairment, whereas high fish consumption tended to be inversely associated with cognitive impairment and cognitive decline (Kalmijn et al., 1997).
3. A survey of the association of cognitive impairment with educational, demographic, and nutritional variables in 3628 older hospitalized people (Cattin et al., 1997) showed that 29% were classified as having cognitive impairment. Moreover, cognitive impairment decreased with increasing body mass index, cholesterol serum level, circulating lymphocytes, and serum albumin, with a gradient of influence for each variable.

In general, these results agree with those of others indicating that intakes of different nutrients or the consumption of a more global diet is associated with better cognitive function in the elderly. Furthermore, the study by Kalmijn et al. (Kalmijn et al., 1997) raises the possibility that high linoleic acid intake is positively associated with cognitive impairment and high fish consumption is inversely associated with cognitive impairment.

Glucose

The actions of glucose in the brain may be related to its role as a precursor for the formation of acetylcholine and many other neurotransmitters. It has been suggested that glucose is also important because many cognition-enhancing substances produce their effect by means of increased glucose utilization (Wenk, 1989). Nootropic drugs (i.e. piracetam and its many analogues), amphetamine and vasopressin stimulate the adrenals and hence increase blood glucose and subsequently the availability and uptake of glucose in the brain. This is suggested by the observations that some substances, particularly those that do not cross the blood-brain barrier (BBB), are ineffective after adrenalectomy. Furthermore, cognitive function, i.e. learning and memory, is correlated with glucose regulation in aged animals and humans (Benton and Sargent, 1992; Foster et al., 1998). The memory-enhancing effect of glucose administration in healthy young adults may be greatest in tests of long-term verbal recall. Glucose may enhance retention in or retrieval from long-term verbal memory (Foster et al., 1998). It was recently demonstrated that cognitive performance, especially memory recall, is ‘fuel-limited’ and can be differentially augmented by increasing the availability of oxygen: the brain’s metabolic resources (Moss et al., 1998). Considering the dependency of glucose metabolism upon oxygen supply, a study of their combined administration (Winder and Borrill, 1998) showed support for the enhancing effects of oxygen (but not for glucose) on delayed recall.

Hypoglycaemia challenge in 14 healthy young and older men showed that older men are prone to more severe cognitive impairment during hypoglycaemia than younger men and are less likely to experience previous warning symptoms if blood glucose falls (Matyka et al., 1997). In three experiments the awareness of falling blood glucose levels during cognitive task performance was demonstrated in healthy young adults, showing that falling blood glucose levels were associated with feeling less energetic (Owens et al., 1997).

Caffeine

As early as 1901, the effects of caffeine on cognition were studied in humans. Improved comprehension and greater speed and accuracy in information processing, particularly evident under conditions of fatigue, were noted (Markowitsch, 1992). We now know that adenosine-A1 antagonism is assumed to be the most important mechanism for explaining the effects of caffeine on human cognition (Nehlig et al., 1992; Stavric, 1992), because caffeine-induced antagonism of adenosine-induced inhibition of noradrenaline, acetylcholine, serotonin and dopamine explains their increased turnover. In human models of age-associated cognitive dysfunction and fatigue, caffeine showed a diverse pattern of results. Whereas the pro-cholinergic effect of caffeine has previously been demonstrated in humans using the scopolamine model of anticholinergic-induced cognitive dysfunction (Riedel et al., 1995a), the pro-noradrenergic effects of caffeine were recently demonstrated in a similar manner showing that caffeine reversed clonidine-induced cognitive impairment in humans (Brice et al., 1998). It was also shown that the beneficial effects of caffeine in low mental load conditions cannot be attributed to reduced distractibility or increased suppression of task-irrelevant response tendencies (Kenemans and Verbaten, 1998). Caffeine also impaired complex cognitive performance in the hypoxia and hyperventilation model of cognitive dysfunction, although it was shown that these effects were not interfering with the challenges per se, but appeared to be associated with the stressfulness of the procedure and hence could be explained in terms of overarousal (Hogervorst et al., 1998a). Low doses of caffeine, however, improved memory and also a wide range of cognitive functions after strenuous endurance exercise, when the arousal level was quite high. The cognition-enhancing effects of caffeine could not be explained by vigilance-enhancing effects before exercise or restoration of deteriorated psychomotor and cognitive performance due to fatigue after exercise (Hogervorst et al., 1999). It was also recently demonstrated that tea, despite lower caffeine levels, produces similar alerting effects to coffee, but is less likely to disrupt sleep (Rigney et al., 1998). In a study investigating the effects of caffeine in different age groups (Hogervorst et al., 1998b), no effects on cognitive performance were found either in young or elderly individuals, but an improvement was shown in middle-aged individuals, which was attributed to the higher daily caffeine intake in the middle-aged group. Rogers and Dernoncourt (Rogers and Dernoncourt, 1998) concluded that there is little unequivocal evidence to show that regular caffeine use is likely to benefit mood or performance substantially, and that one of the significant factors motivating caffeine consumption appears to be the relief of its withdrawal. This conclusion seems to be substantiated by a double-blind placebo-controlled cross-over study of the ingestion of placebo or caffeine 3 times daily for 6 days followed by a 7th [challenge] day of placebo or caffeine ingestion (James, 1998). No evidence was found that caffeine improved performance, either in the context of acute or habitual use. On the contrary, performance was found to be significantly impaired when caffeine was withdrawn abruptly following habitual use. Participants reported feeling more alert and less tired following acute ingestion of caffeine, but feeling less alert in conjunction with chronic exposure to the drug. In addition, caffeine withdrawal was associated with reported increases in frequency and severity of headache, and with reports of sleeping longer and more soundly (James, 1998). Whatever caffeine's effects on cognitive performance, they are not easily explained in terms of the simple arousing effects of caffeine, although many authors believe that the beneficial effects of low doses of caffeine are most easily demonstrated when alertness is decreased, and that increased turnover of central monoaminergic neurotransmitters

may explain at least some of the improvements produced by caffeine in states of low arousal. Furthermore, the beneficial effects of caffeine seem to depend on its amount of habitual use (James, 1998; Rogers and Dernoncourt, 1998), which has been shown to be highest in middle-aged people (Hogervorst et al., 1998b; Riedel et al., 1995b).

Tryptophan depletion

Recently, there has been an explosion of studies into the serotonergic determinants of human cognition. Acute tryptophan depletion (ATD) lowers serotonin (5-hydroxytryptamine; 5-HT) levels in the brain by lowering 5-HT synthesis via depletion of its precursor, tryptophan, through administration of an amino acid mixture without tryptophan but with a high content of large neutral amino acids (LNAAs) and other amino acids. The mixture stimulates protein synthesis requiring tryptophan. Therefore, free tryptophan in the circulation is used. There is competition at the level of the BBB between tryptophan and the LNAAs. The uptake of plasma tryptophan in the brain is strongly associated with the ratio of tryptophan to LNAAs. Because of the large amount of competing LNAAs, less tryptophan will cross the BBB. In humans, the ingestion of 50-100 g of an amino acid mixture without tryptophan leads to a 75-90% reduction in plasma tryptophan within 4-6 h (Young et al., 1985). A subsequent decline in the rate of brain 5-HT synthesis has also been shown in human positron emission tomography brain scans (Nishizawa et al., 1997).

Most studies describing the cognitive effects of ATD have not even been published yet, but merely have been presented at recent conferences. However, because we consider this field of high potential relevance for the relation between nutrients and cognitive function, the state of the art will be briefly described here.

ATD has been reported to impair learning and long-term memory consolidation in healthy young individuals (Park et al., 1994; Riedel et al., 1999; Rowley et al., 1998; Schmitt et al., 1998; Shansis et al., 1998), elderly individuals and AD patients (Porter et al., 1998), to impair decision making (Rogers et al., 1997), and to impair and alter various other indices of cognitive performance such as proofreading (Danjou et al., 1990; Young et al., 1985), and focused attention (Coull et al., 1995; Rowley et al., 1998; Schmitt et al., 1998). ATD effects have been shown to be specifically related to a lowering of tryptophan rather than to altered protein synthesis (Klaassen et al., 1997). ATD-induced effects resemble impaired ventromedial prefrontal cortex functioning associated with disrupted monoaminergic orbito-frontal striatal circuitry (Rogers et al., 1997). Recent positron emission tomography scan data showed that ATD in recovered depressive patients specifically impaired activity in brain regions such as the anterior cingulate and orbitofrontal cortex, and suggested that the anterior cingulate cortex is a critical region that represents the neural substrate for the expression of depression-related impaired cognition (Smith et al., 1998).

Two aspects are of particular interest in ATD and cognition. First, the method, which is basically a nutritional manipulation with a definite pharmacological effect on the brain and consequently cognitive functioning, may provide insights into the probable common biological (i.e. serotonergic) basis of vulnerability to depression and cognitive disorders, both of which incidences increase with age. Second, the method may provide more insight into the positive

and negative effects of dietary factors on cognitive functions, because ATD may mimic a low dietary carbohydrate/protein ratio (Wurtman and Wurtman, 1988).

Conclusion

Many nutrients or indices of nutritional status are associated with cognitive functioning, although the size of the effects on cognitive performance may be small and not much is known about their mechanisms of action. Even within the few interventional studies reviewed, however, results can be interpreted as promising for the place of nutritional supplements in the treatment of age-associated cognitive decline and dementia. Studies with ginkgo biloba and α-tocopherol yield results that do not seem to differ in terms of effect size when compared with clinical trials of cholinesterase inhibitors such as tacrine (Knapp et al., 1994) and donepezil (Bryson and Benfield, 1997). In vitamin studies, further evidence seems to accumulate that folate, rather than vitamin B12 status is associated with cognitive function. A proper dietary composition with regard to the ratio of carbohydrates to proteins, as well as the inclusion of sufficient micronutrients, seems to be favourable in the maintenance of cognitive function in the elderly. At present, there is not much evidence to support the role played by caffeine in enhancing cognition in elderly people. In general, there appears to be a limited amount of research in the area of nutrients and cognitive function in humans, particularly the elderly.

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3

Nutrients, Age and Cognitive Function: Part II

1998-2001

Our knowledge about the influence of nutritional supplements on human cognition, especially in the elderly, rests largely on animal behavioral research and neurochemical experiments in vitro, while only a few epidemiological studies and even fewer controlled experiments in humans are reported. This is an inherent problem, due partly to the difficulty of conducting controlled nutritional experiments in humans, but may also partly be due to the gap between the research disciplines of nutritional and neurobehavioral experimental science. Learning objectives— The aim of this paper is to discuss some new findings in this line of research, and to stress the importance of the need to start bridging the gap between disciplines by identifying possible human experimental models of altered cognitive function, which can elucidate the specific mechanisms of action through which nutritional supplements may enhance cognitive performance in humans in vivo. These experimental models are important because the research in this field is mostly based on epidemiological studies, which describe associations between nutrients and cognitive functions. Contrary to epidemiological studies, experimental models mimic associations between nutritients and cognition by manipulating their presumed mechanisms of action and can eventually explain the causal nature of found associations.

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Introduction

During the search for cognitive enhancing nutritional supplements, one can not ignore comparing efficacy and side effects of nutritional supplements with registered medicines. In most western European countries and the US, the first three medicinal drugs have recently been registered for the indication of Alzheimer's Disease (AD). These concern the cholinesterase inhibitors tacrine (Knapp et al., 1994), donepezil (Bryson and Benfield, 1997) and rivastigmine (Jann, 2000). Furthermore, clinical trials are now, just as we predicted 5 years ago (Riedel and Jolles, 1996), moving from AD towards investigating the pharmacological treatment of the less severe condition of mild cognitive impairment (MCI). Whilst one would demand more clinical efficacy and tolerate more side effects of medicinal drugs if the disease condition is more severe, such as in AD, one would tend to accept less side effects of nutritional supplements in MCI. However, one would still demand proof of efficacy and as few side effects as possible. This is precisely where the need for demonstrating the efficacy of nutritional supplements comes in. If effective nutritional supplements or optimally composed diets could be identified, one would prefer nutritional treatments over prescription medicine. This is simply because less side effects are expected in nutritional treatments that improve or at least maintain optimal cognitive performance in adulthood and ageing. Some researchers argue that the concept of MCI – or memory complaints in elderly people - is a stage of normal ageing, whereas others see it as an early stage of dementia (Jonker et al., 2000). We found a higher frequency of the ApoE4 genotype (37.8% with at least one E4 allele) in 119 elderly subjects with MCI who fulfilled the criteria for age-associated memory impairment (AAMI), when compared to 87 matched controls (22%) (Jorissen et al., 2001). The frequency of at least one E4 allele of the AAMI population is in between the control population and the allele frequency of approximately 45% in Alzheimer's Disease populations in other studies. The frequency of the ApoE4 genotype may be objective evidence of memory impairment in AAMI. This places AAMI somewhere between normal ageing and dementia of the Alzheimer's type.

In a recent review of epidemiological and experimental studies, we concluded that several nutrients -such as beta-carotene, alfa-tocopherol, Folate rather than vitamin B12 and Ginkgo biloba - or indices of nutritional status are associated with cognitive functioning (Riedel and Jorissen, 1998). The magnitude of nutrient effects on cognitive performance was considered to be small but not to be very different from that of registered medicinal or investigational cognition-enhancing or anti-dementia drugs. In this paper, we will shortly review results of recent research (i.e. 1998-2001) into the influence of nutrients on cognitive functioning, and highlight possible human experimental models, which might be used in future studies to elucidate the brain mechanisms underlying nutrient-induced effects on cognitive performance.

Recent findings

Vitamins

No association was found between self-reported antioxidant nutritional supplement use (vitamin A, C, E, beta-carotene, zinc, or selenium) and cognitive function after correction for age, education and gender (Mendelsohn et al., 1998). In AD patients, lowered plasma levels of vitamin C were found to be proportionally related to the degree of cognitive decline (Rivière et al., 1998). The authors contended that the nutritional status of these patients was

normal, hence the declined concentrations of vitamin C could be due to oxidative stress associated with the disease. Smith et al. (Smith et al., 1999) showed in a double-blind placebo-controlled study in 205 (110 female and 95 male) elderly subjects, that a 12 month antioxidant supplementation, including beta carotene, alpha-tocopherol and ascorbic acid, had little effect on mental performance. There were only 4 significant differences of 117 analyses, and these could have been expected by chance. Morris et al. (Morris et al., 1998) studied the association between use of vitamin E and vitamin C and the incidence of Alzheimer's Disease in a prospective study of 633 volunteers of 65 years and older. The results indicated that none of the 27 vitamin E supplement users and none of the 23 vitamin C supplement users had Alzheimer's Disease after a follow-up period of 4.3 years. Based upon the incidence of the nonusers, corrected for age, sex, education and follow-up interval, an incidence of 2.5 and 3.2 for the E- and C-users respectively would be expected. This suggests that use of vitamin E and vitamin C supplements may lower the risk of Alzheimer's Disease. The Honolulu-Asia Aging Study (Masaki et al., 2000) did not show a protective effect of Vitamin E and C supplements of Alzheimer's Disease. This study showed a protective effect of these vitamins for vascular dementia and for mixed/other dementia in 3,385 men who used vitamin E and C supplements in 1988 during a dementia prevalence survey carried out in 1991 to 1993. However there was a positive association between use of either vitamin E or C supplements in 1988 and cognitive test performance between 1991 and 1993. The authors suggest that vitamin E and C supplements may protect against vascular dementia and may improve cognitive function in the elderly. Previous studies suggested that low concentrations of folate are related to poor cognitive function and to neurodegeneration as in dementia. A report from the so called 'nun study' concluded that among 30 elderly Catholic sisters who lived in one convent, ate from the same kitchen, and were highly comparable for a wide range of environmental and lifestyle factors, low serum folate was strongly associated with atrophy of the cerebral cortex (Snowdon et al., 2000). Homocysteine on the other hand has been associated with reduced cognitive function. However, no support was found for this association in a random sample of 702 community-dwelling respondents aged 55 years or over (Kalmijn et al., 1999). To determine if long-term, high-dose vitamin supplementation could reverse cognitive malfunction in older people, a longitudinal study was performed in 20 non-vitamin-deficient elderly females with a Folstein mini mental state examination score indicating cognitive malfunctions, relating the 12-month outcome to baseline values. No improvement in cognitive malfunction was noted despite elevation of blood vitamins levels. Administration of a high-dose vitamin and mineral supplement for 1 year did not improve cognitive malfunction in non-vitamin-deficient elderly in this study (Baker et al., 1999). In a study with 70 elderly male subjects, lower concentrations of vitamin B12 and folate and higher concentrations of homocysteine were associated with poorer spatial copying skills (Riggs et al., 1996).

Recently, McCaddon et al. (McCaddon et al., 1998) showed that thirty patients suffering from senile dementia of the Alzheimer type had significantly higher plasma levels of homocysteine than thirty healthy elderly controls. Homocysteine and vitamin B12 plasma levels were interrelated with cognitive scores. In another study, higher plasma concentrations of homocysteine were also found in patients with vascular dementia or minor cognitive impairment and also in patients with senile dementia of the Alzheimer type (Lehmann et al., 1999). Vitamin B12 and folate are both involved in the conversion from homocysteine to methionine (Hunt and Groff, 1990), which is indirectly involved in the maintenance of the myelin sheath. This

mechanism might explain the association between the higher plasma concentrations of homocysteine, the lower concentrations of vitamin B12 and folate, and reduced cognitive function. The high homocysteine plasma concentrations might on the other hand influence cognitive performance in the elderly, due to it's excitotoxicity. High concentrations of homocysteine may induce high concentrations of homocysteic acid and cysteine sulphinic acid, which act as endogenous agonists of NMDA receptors (Parnetti et al., 1997). It has been shown that supplementation with a mixture of B-vitamins with or without antioxidants lowered homocysteine concentrations compared to placebo in a randomised double-blind placebo-controlled trial of 132 healthy men (Woodside et al., 1998), whereas antioxidants alone induced a non-significant increase of homocysteine concentrations compared to placebo. This decrease of homocysteine concentrations, induced by supplementation of B-vitamins, is a promising finding that might trigger researchers to further explore the influence of vitamin B12 and folate supplementation on cognitive performance. Recently, Ravaglia et al. found no relationship between homocysteine, B vitamins and reduced cognitive function, measured with the Mini Mental State Examination (MMSE) and a battery of neuropsychological tests, in 56 healthy cognitively normal elderly subjects (Ravaglia et al., 2000). In another recent study with 156 elderly volunteers Budge et al. (Budge et al., 2000) showed that the cognitive performance scores on the cognitive section of the Cambridge Examination for Mental Disorders of the Elderly were inversely related to homocysteine concentrations, which was independent of age, gender, IQ and depression. However they found no association between homocysteine concentrations and Mini Mental State Examination (MMSE) scores. According to the authors this last result may be caused by the small number of volunteers. The results of the above mentioned vitamin studies are not consistent, which makes it difficult to draw a conclusion about the neuroprotective effects of vitamins at this stage. The anti-oxidative effects of vitamin E and C supplements and the effects of the B vitamins on cognitive function need to be confirmed in long-term placebo-controlled studies.

Nutritional Status

In a study into diet composition and cognitive function in elderly males in Finland, Italy and The Netherlands the 'Health Diet Indicator' (HDI), defined according to the guidelines of the World Health Organisation (WHO), was used. A higher HDI means that a person's diet is more in accordance with the WHO guidelines. In 4 of the 5 cohorts that were studied a lower prevalence of cognitive decline (MMSE <24) was associated with an increased HDI (Huijbregts et al., 1998). This indicates the importance of the effects of a nutritionally healthy diet.

Wine and other alcohol containing beverages

Interesting reports of epidemiological studies into the association of wine consumption with the incidence of AD have been reported. A 4-fold lowered relative risk for AD was found in subjects categorized as moderate drinkers (3-4 glasses wine / day), compared to non-drinkers (Orgogozo et al., 1997). These results could not be confirmed in another study, because when the data were corrected for whether or not subjects were living in a nursing home, the association disappeared, putatively because wine consumption in elderly nursing homes is kept low (Leibovici et al., 1999). A possible mechanism for the protective effect of wine is provided by the antioxidative properties of polyphenoles which are most prominent in red wine. These effects are independent of alcohol and can also occur in alcohol-free red

wine (Serafini et al., 1998). In the aforementioned epidemiological studies the difference between consumption of white and red wines has however not been taken into account.
Not only has the association between wine consumption and the incidence of AD been studied. The relationship between alcohol consumption, which includes all types of beverages, and cognitive performance has recently been studied by several researchers. Launer et al. (Launer et al., 1996) showed in the Zutphen Elderly Study that men with cardiovascular disease or diabetes and low-to-moderate alcohol intake had a significantly lower risk of poor cognitive function, which was measured in 1990 and 1993 with the Mini-Mental State Examination, compared to abstainers. In the Framingham Heart Study the association between alcohol consumption and cognitive performance was analysed separately for men and women, since the researchers expected a different alcohol-cognition relationship for male and female drinkers (Elias et al., 1999). Test performance of moderate (>2 and $\leq$ 4 drinks/day) male drinkers performed significantly better than abstainers only on logical memory delayed-recall, whereas heavy (>4 and $\leq$ 8 drinks/day) drinkers performed better on logical memory delayed-recall, on the attention and concentration (AC) composite score, and on total composite score. Female drinkers showed superior performance compared to abstainers on more cognitive tests than male drinkers. Light (1-2 drinks/day) female drinkers performed better on logical memory delayed-recall, on the learning and memory (LIM) composite and the total composite, whereas moderate female drinkers scored significantly better than abstainers on delayed memory, word fluency, similarities and on the AC, LIM and total composites.
Thus moderate wine consumption might not only be associated with a lowered relative risk for AD, but light to moderate alcohol consumption might also influence cognitive performance. Experimental studies are necessary to confirm these associations.

Herbal extracts

The ingredients of ginkgo biloba, flavonoïds, terpenoïds and organic acids, are putative antioxidants as well. The influence of chronic oral treatment with 240 mg/day of Ginkgo biloba on the clinical course of AD was investigated in a 3 month, double-blind, randomised, placebo-controlled parallel-group design in 20 outpatients. A small improvement of cognitive performance was found. However, there was no effect on the Alzheimer's Disease Assessment Scale (ADAS) cognitive and non-cognitive subscales (Maurer et al., 1997). In 1992 a meta-analysis was conducted in which the authors concluded that 8 out of 40 studies of ginkgo biloba for cerebral insufficiency were of adequate methodology, but all 8 well-controlled studies found positive effects of ginkgo (Kleijnen and Knipschild, 1992). A remarkable ginkgo study was then carried out by this group in The Netherlands. In over 250 elderly subjects diagnosed with MCI, vascular dementia, or AD, no effects of ginkgo on a large number of cognitive tests and clinical scales were found in a double-blind, placebo-controlled, parallel-groups study (Van Dongen, 1999). One of the explanations for the lack of effect of ginkgo in this very thoroughly conducted trial was that this was the first ginkgo study employing a truly identical placebo (Knipschild et al., 1998). Consequently the authors assertion was that most, if not all other results of previously conducted clinical trials on ginkgo biloba are based on expectancy effects (Van Dongen, 1999). Another popular herbal preparation from ancient China, ginseng, is also said to enhance cognitive function. Only 4 placebo-controlled trials have been carried out in healthy volunteers so far. In three studies Panax ginseng improved performance on tasks of mental arithmetic and abstract thinking, while eleuthero ginseng improved memory performance. Whether ginseng can actually en-

hance cognition in the elderly remains to be established (Vogler et al., 1999). Wesnes and colleagues (Wesnes et al., 2000) recently studied the cognitive effects of a combination of Ginkgo biloba and Panax ginseng in 256 healthy middle-aged subjects. Dosages of 160 mg b.i.d. or 320 mg o.d. were tested in a placebo-controlled, double blind, parallel group 14-weeks study. The combination Ginkgo biloba / Panax ginseng improved significantly the Quality of Memory Index compared to placebo. The Quality of Memory Index is a combination of cognitive tests for the quality of episodic and working memory. The treatment did not influence power of attention, speed of attention and the speed of memory processes. These Ginkgo Biloba results are promising, but more chronic dosage studies are necessary to replicate these cognitive enhancing findings. The quality of the placebo capsule should especially be taken into account.

Lipids

The cognition enhancing potential of phospholipids has been studied in AD patients and in subjects with MCI. About a decade ago, a number of placebo-controlled studies were carried out with bovine cortex phosphatidylserine (PS) which was shown to improve memory in subjects with age-associated memory impairment. PS has a function in the maintenance of the membrane structure of nerve cells, thereby maintaining the conduction of nerve cells. In ageing the concentration of PS is reduced. Since the recent genesis of plant-derived soy-PS, the interest for PS as a nutritional supplement has been revived (Pepeu, 1999). Recent animal studies show that soy-PS improves aspects of memory in rats in a comparable manner with bovine PS (Blokland et al., 1999). In a recently conducted published placebo-controlled study into the efficacy of soy-PS in the treatment of subjects, fulfilling the criteria of age-associated memory impairment, 120 male and female volunteers of 58 years and older received a 12 weeks treatment of 300 mg soy-PS, 600 mg soy-PS or placebo. A neuropsychological test battery, including the visual verbal learning test, the memory scanning test, the fluency test, the Stroop colour word test, the signal detection test, the motor choice reaction time test, the concept shifting and the tower of London, was conducted at baseline, after 6 and 12 weeks of treatment and after a washout period of 3 weeks. Both dosages of Soy-PS did not influence learning and memory, choice reaction time, planning and attention (Jorissen et al., 2001). According to the authors the fatty acid content of PS is an important factor which should be controlled for in future studies. The fatty acid content of the PS-formulas used in animal studies differed considerably, whereas the fatty acid content is not mentioned in most human studies. This fatty acid aspect is rather important, since the administration of n-3 and n-6 essential fatty acids in a 1:4 composition has been said to improve cognition as well. A placebo-controlled study in 100 AD patients (60 treatment and 40 placebo) of α-linolenic acid (n-3 polyunsaturated fatty acid; PUFA) and linolenic acid (n-6 PUFA) reported a positive effect on mood, appetite, sleep and short term memory (Yehuda et al., 1996). This fatty acid treatment might influence cognitive functions through the modulation of neuronal membrane fluidity (Yehuda et al., 1999). The relation between cognitive functions and daily fatty acid intake has also been addressed in epidemiological studies. According to Ortega et al. (Ortega et al., 1997) adequate cognitive functioning in elderly (assessed with the MMSE) was associated with lower consumption of monounsaturated fatty acids (MUFA), saturated fatty acids (SFA) and cholesterol. However, in another study cognitive function was positively associated with consumption of MUFA, 80% of which were consumed through constituents of olive oil (Solfrizzi et al., 1999). According to the authors, the

protective effect of MUFA could especially be associated with the antioxidant action of tocopheroles and polyphenoles in olive oil.
Results from the Rotterdam Study (Kalmijn, 2000) showed that high intakes of total fat, saturated fat and cholesterol were associated with an increased risk of dementia, whereas an inverse association between fish consumption, an important source of n-3 PUFAs, and dementia was found.
The association between cholesterol levels and cognitive function has also been reported. Since high serum cholesterol levels seems to be associated with better cognitive function in two epidemiological studies (Benton, 1995; Muldoon et al., 1997), Wardle et al. (Wardle et al., 2000) studied the effects of cholesterol lowering dietary treatments on mood and cognitive functioning in 176 adults with raised serum cholesterol levels. In this study two cholesterol lowering diets did not influence psychological well-being (mood and aggression), or the cognitive measures motor speed, memory and choice reaction time after 6 and 12 weeks of treatment. However sustained attention was relatively impaired in the treated groups compared to the control group. In another study the psychological effects of treatment of hypercholesterolemia with lovastatin were investigated (Muldoon et al., 2000). The placebo-treated subjects showed a greater improvement on attention and psychomotor speed, compared to the lovastatin-treated subjects. The cholesterol-lowering treatment did (also in this study) not influence psychological well-being. These are very interesting results, since the studies show the same impairment of attentional functions after a cholesterol-lowering treatment, which is in contrast with the mentioned association between adequate cognitive functioning and lower consumption of cholesterol and other lipids (Ortega et al., 1997). At this point it is unclear whether the association between cholesterol level and cognitive performance is determined by change of cholesterol level or by the levels itself.

Amino acid and protein / carbohydrate diet manipulations
Serotonin syntheses in the brain depends on the tryptophan (TRP) concentration in the central nervous system. The ratio TRP to Large Neutral Amino Acids (LNAA's) is important, because TRP competes with the other LNAA's to pass the blood-brain barrier. Therefore, more serotonin will be synthesised in the brain not only when TRP increases but also when the total concentration of the other LNAA's decreases. Food manipulations achieving subchronic relative supplementation or depletion of TRP are a carbohydrate-rich protein-poor diet (CR/PP) and a protein-rich / carbohydrate-poor diet (PR/CP) respectively (Markus et al., 1998). In stress-prone subjects, CR/PP prevented a deterioration of mood and performance under uncontrollable laboratory stress conditions. The assumption was that in stress-prone subjects (HS) there is a higher risk of serotonin (5-hydroxytryptamine; 5-HT) deficiency in the brain. Consequently, carbohydrates may prevent a functional shortage of central 5-HT during acute stress, due to their stimulatory effect on brain TRP. Significant increases were found in the ratio of TRP to Large Neutral Amino Acids (LNAA) during the CR/PP diet compared with the PR/CP diet. With respect to cognitive performance, significant dietary effects were found on speed of memory scanning. It is suggested that CR/PP food in HS subjects may increase personal control, probably under the influence of higher levels of brain TRP and 5-HT (Markus et al., 1999; Markus et al., 1998). In another study administration of alfa-lactalbumin, which is a bovine protein with a high TRP content, increased the plasma Trp/ΣLNAA ratio, reduced cortisol and improved mood under stress in stress-vulnerable subjects (Markus et al., 2000).

The daily administration of tyrosine, a large neutral amino acid found in dietary proteins, which has received recent attention as a potential treatment for stress, has been shown to be effective in enhancing cognitive function in healthy young individuals who were physically exhausted through military training during 5 days. Supplementation with tyrosine may reduce the effects of psychosocial- and physical stress and fatigue on cognitive task performance (Deijen et al., 1999). Another study indicated that tyrosine may sustain working memory in situations of high mental load (Thomas et al., 1999).

Human Experimental Models

Experimental studies are necessary to confirm the association between different nutrients and cognitive performance in the elderly. Human experimental models of altered cognitive function, might indicate the specific mechanisms of action through which the nutrients or nutritional supplements may enhance cognitive performance. Therefore we will now move on to briefly discuss possible human experimental models for the mentioned nutrients.

Several of the nutrients mentioned above, including the vitamins, polyphenoles in wine, tocopheroles and polyphenoles in olive oil, and flavonoids, terpenoids and organic acids in ginkgo biloba, display antioxidative properties. These antioxidants may prevent oxidative stress by acting as free radical scavengers. The hypoxia model may be a useful human experimental model to study the antioxidative actions of these nutrients. During the hypoxia condition subjects breath low-oxygen air, either through pressurised gas tubes within a normal environment, or in low pressure chambers simulating the effects of high altitude. Subsequently, controlled experiments on the acute or chronic effects of substances (e.g. antioxidants) with neuroprotective properties are carried out (Saletu and Grünberger, 1984).

Phospholipids and fatty acids influence the functioning of the neuronal membranes. By altering the membrane fluidity, membrane receptor formation and function, membrane signalling, and surface membrane activity they affect the activity of the blood-brain barrier, neurotransmitters, hormones and cytokines. These effects cannot be measured in humans directly, but have to be deduced from altered measures of brain function and cognition. The administration of anaesthetic gases to human volunteers is a specific model of neuronal membrane dysfunction with its concomitant impairment of human cognition.

There are also nutrient-based human experimental models of impaired neurotransmission. Acute tryptophan depletion (ATD) might be a human experimental model to study the efficacy of nutrients or nutritional supplements which influence cognitive performance through a serotonergic pathway. During this experiment volunteers drink different amino acid mixtures on two separate test days. The tryptophan depletion mixture contains a mixture of amino acids including all long chain neutral amino acids except tryptophan. This mixture decreases plasma tryptophan concentration and consequently brain tryptophan concentrations and serotonin synthesis. The brain tryptophan decrease is probably caused by two mechanisms. The amino acids administered in the mixture stimulate protein synthesis at tissue level, which causes a decrease of plasma tryptophan, and on the other hand, tryptophan competes for the same carrier enzyme system as the other LNAA’s to pass the blood-brain barrier (Fadda, 2000). It has been shown that elderly and Alzheimer’s Disease patients are more sensitive to the memory impairing effects of ATD (Porter et al., 2000). Consequently, if nutritional substances reverse or attenuate ATD-induced memory impairment, this can be taken as an indication of their serotonergically mediated cognition enhancing efficacy in elderly.

Acute phenylalanine/tyrosine depletion (APTD) is a comparable model that reduces dopamine function, and has similar mood lowering (Leyton et al., 2000), and memory impairing effects as in ATD (Harmer et al., 2001). Likewise, if nutritional substances reverse or attenuate APTD-induced cognitive impairment, this can be taken as an indication of their dopaminergically mediated cognition enhancing efficacy in elders.

Concluding remarks

In epidemiological studies associations have been established between nutritional status, consumption of vitamins, wine, fatty acids and cognitive decline. This does not mean however, that depletion of certain nutrients can actually be suppleted through a change in diet or through the use of nutritional supplements. It seems reasonably well established for example that low folate signals MCI, but this does not necessarily mean that MCI or AD can be prevented or postponed by ingestion of folate. The most important question is therefore, whether these manipulations actually halt or decelerate cognitive decline or even enhance cognitive functions. Moreover, it is difficult to compose a diet in which all putatively cognition enhancing nutrients are properly taken into account. A proper dietary composition as to the ratio of carbohydrates to proteins as well as the inclusion of sufficient micronutrients seems to be favourable to the maintenance of cognitive function under stress and putatively at older age. In general, there appears to be a limited amount of controlled studies in the area of nutrients and cognitive function in human subjects, particularly in the elderly.

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4 The Influence of Soy-derived Phosphatidylserine on Cognition in Age-associated Memory Impairment

Phosphatidylserine (PS) is a phospholipid widely sold as a nutritional supplement. PS has been claimed to enhance neuronal membrane function and hence cognitive function, especially in the elderly. We report the results of a clinical trial of soybean-derived PS (S-PS) in aging subjects with memory complaints.

Subjects were 120 elderly (>57 years) of both sexes who fulfilled the more stringent criteria for age-associated memory impairment (AAMI); some also fulfilled the criteria for age-associated cognitive decline. Subjects were allocated at random to one of the three treatment groups: placebo, 300 mg S-PS daily, or 600 mg S-PS daily. Assessments were carried out at baseline, after 6 and 12 weeks of treatment, and after a wash-out period of 3 weeks.

Tests of learning and memory, choice reaction time, planning and attentional functions were administered at each assessment. Delayed recall and recognition of a previously learned word list comprised the primary outcome measures.

No significant differences were found in any of the outcome variables between the treatment groups. There were also no significant interactions between treatment and 'severity of memory complaints'.

In conclusion, a daily supplement of S-PS does not affect memory or other cognitive functions in older individuals with memory complaints.

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Introduction

Cognitive aging, age-associated cognitive decline, and mild cognitive impairment are all defined as the decline of cognitive functioning with age. The aging of the population results in an increasing prevalence of diminished cognitive functioning, and widespread requests for therapeutic agents to attenuate cognitive decline. Despite worldwide concern about the increasing problem of cognitive decline in the aging population, not much research has been devoted to empirically studying the influence of nutritional supplements on human cognitive functioning, particularly in the elderly. There are few placebo-controlled studies of nutritional supplements in the elderly in the recent literature (Riedel & Jorissen, 1998). Nonetheless, many nutritional supplements are advocated on the grounds that they might enhance cognitive function, especially in the elderly. One such example, phosphatidylserine (PS), is a naturally occurring phospholipid, the daily intake of which is about 0.1 mmol (approximately 75 mg) in human diet (Bruni et al., 1989). PS can be derived from several sources. Bovine cortex PS (BC-PS) has until recently been used in all PS efficacy studies. However, because of the obvious safety considerations for products of animal origin, alternative sources of PS have been developed, for example, soybean (S-PS) or eggs (E-PS).

PS influences the functioning of neuronal membranes, for example, the release of vesicles containing neurotransmitters from the presynaptic terminal (Nishizuka, 1984), signal transduction (Nishizuka, 1984), cell to cell communication, preservation of the cellular sodium-potassium and calcium-magnesium balance (Toffano, 1987) and regulation of cell growth (Nunzi et al., 1990). In addition PS can restore the diminished acetylcholine release seen in cortical slices obtained from aging rats (Vannucchi & Pepeu, 1987). PS is also capable of counteracting scopolamine-induced amnesic effects in rats (Furushiro et al., 1997; Zanotti et al., 1986). This implies that PS may affect the cholinergic system. Finally, it has been shown that BC-PS elevates the reduced NMDA-receptor density in aged mice by approximately 25% (Cohen & Muller, 1992), and generates an increase in the efficacy of hippocampal synaptic transmission (Borghese et al., 1993). Since the content of PS, expressed as percentage of total phospholipids, is higher in human brain than in other human tissues, and this percentage decreases with age (White, 1973), the administration of PS may be beneficial in preventing and treating cognitive impairment in the elderly. This is consistent with ‘the membrane hypothesis of aging’ (Sun & Sun, 1979), which states that age-related changes in the molecular structure and lipid-protein interactions of biological membranes in the central nervous system may impair their function. These changes may thus contribute to acceleration of the cognitive aging process.

Double-blind, placebo-controlled studies showed that BC-PS improved cognitive function in Alzheimer patients (Amaducci, 1988; Delwaide et al., 1986; 1989), in senile mentally deteriorated patients (Palmieri et al., 1987), and in subjects with mild to severe cognitive deterioration (Cenacchi et al., 1993; Villardita et al., 1987) such as in Age-Associated Memory Impairment (AAMI) (Crook, 1998; Crook et al., 1991; Gindin et al., 1993). The diagnostic term AAMI refers to complaints of memory impairment in tasks of daily life. People with AAMI also have an impaired performance on psychological tests (Crook et al., 1986). On the basis of these double-blind studies and several open trial studies (Allegro et al., 1987; Caffarra & Santamaria, 1987; Granata, 1987; Sinforiani et al., 1987), it has been suggested that BC-PS is effective in the treatment of cognitive impairment in the elderly. Crook and colleagues

(1991) found improvement of memory and learning after 12 weeks of treatment with 300 mg BC-PS in a double-blind placebo-controlled study with 149 AAMI subjects. Additional analyses showed that this effect was only present in a subgroup of patients with lower memory performance at baseline.
Since its introduction, only one placebo-controlled study into the cognition-enhancing effects of S-PS has been reported as an abstract (Gindin et al., 1993). In this study with initially 72 AAMI-subjects memory and mood improved significantly in the 300 mg S-PS treatment group compared to placebo. However, in contrast to the findings of Crook et al. (1991), this effect was stronger in a subgroup of subjects with a better memory performance at baseline. These findings at least cast some doubt on the efficacy of PS in AAMI.

The present study evaluated the efficacy of two doses of S-PS (300 mg or 600 mg daily), in a double-blind, placebo-controlled study of subjects with AAMI. The primary objective of this study was to ascertain the possible dose-related enhancing effects of 300 mg S-PS, 600 mg S-PS, compared to placebo on memory and other cognitive functions after 12 weeks of treatment. The secondary objective was to investigate a possible differential effect of S-PS between subgroups of subjects with AAMI because previous clinical studies with BC-PS (Crook et al., 1986) and also of other treatments of AAMI (Israel et al., 1994) have shown that the treatment response is correlated positively to the severity of the cognitive impairment at baseline.

Methods

Subjects

Subjects were recruited through advertisements in the local newspaper and local television, and through posters in general practitioners' waiting rooms and at places where the elderly meet, such as sport or recreation centers for the elderly. Subjects were older than 57. All subjects fulfilled the criteria for AAMI (Crook et al., 1986). They had complaints of memory impairment in every day life (a score of over 24 on the memory complaints questionnaire (MAC-Q) (Crook et al., 1992)) and their memory test performance was at least one standard deviation below the mean established for young adults on at least one of the following standardized tests: Benton Visual Retention Test (Benton, 1975), Logical Memory Subtest of the Revised Wechsler Memory Scale (Wechsler, 1974) or the Paired Associates Learning Subtest of the Revised Wechsler Memory Scale (Wechsler, 1974). Their intellectual function was adequate, as determined by an IQ score equivalent to 90 or more (raw score of at least 32), assessed with the Vocabulary Subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955). They did not suffer from dementia, as determined with the Mini-Mental State Examination (Folstein et al., 1975) (MMSE score > 24; or depression, as assessed with Geriatric Depression Scale (score < 15) (Yesavage & Brink, 1983). The exclusion criteria are listed in table I. A shortened version of the Groningen Intelligence Test (Luteijn & van der Ploeg, 1983) (GIT1), with three subtasks, was used to estimate the IQ score. A subgroup of AAMI subjects also fulfilled the criteria for Age-Associated Cognitive Decline (AACD) (Rediess & Caine, 1996). This AACD subgroup had memory scores (delayed recall at intake) of at least one standard deviation below the norm for their own age group (Houx, 1991).
The AACD subgroup is in this study referred to as the 'moderate' group, and the Non-AACD subgroup is referred to as the 'mild' group. The Medical Ethics Committee of the University Hospital of Maastricht approved the study and all subjects gave written informed consent.

Table I. Exclusion Criteria.

- evidence of delirium, confusion, or other disturbances of consciousness
- any neurological disorder that could produce cognitive deterioration as determined by history, clinical neurological examination, or neuroradiological examination
- history of any infective or inflammatory brain disease
- evidence of significant cerebral vascular pathology as determined by a Hachinski Ischemic Score (HIS) over 4 (Small, 1965)
- history of head injury
- current psychiatric diagnosis according to DSM-IV criteria of a major psychiatric disorder
- current diagnosis or history of alcoholism or drug dependence
- any medical disorder that could produce cognitive deterioration
- use of any psychotropic drug or any other drug that may significantly affect cognitive function during the month prior to psychometric testing
- known hypersensitivity of PS

In all, 546 subjects were recruited, of whom 132 entered the study. Of these, 120 subjects completed the treatment according to protocol (placebo: n=39, 300 mg S-PS: n=40 and 600 mg S-PS: n=41). The treatment groups were not significantly different in IQ, age or sex. The subjects' characteristics at baseline are summarized in table II. Drop-outs were distributed equally over the three groups: 4 in the placebo group, 5 in the 300 mg S-PS group and 3 in the 600 mg S-PS group. The reasons for drop-out were not related to the treatment according to an independent physician.

Table II. Subject characteristics at baseline.

	Placebo	300 mg PS	600 mg PS
Number of subjects	39	40	41
Number of females	19	21	21
Number of males	20	19	20
Number of AACD*	9	18	14
Mean Age (S.E.)	64.6 (0.9)	65.3 (0.9)	65.8 (1.1)
Mean IQ (S.E.)	120.3 (1.9)	115.1 (1.7)	117.7 (1.9)

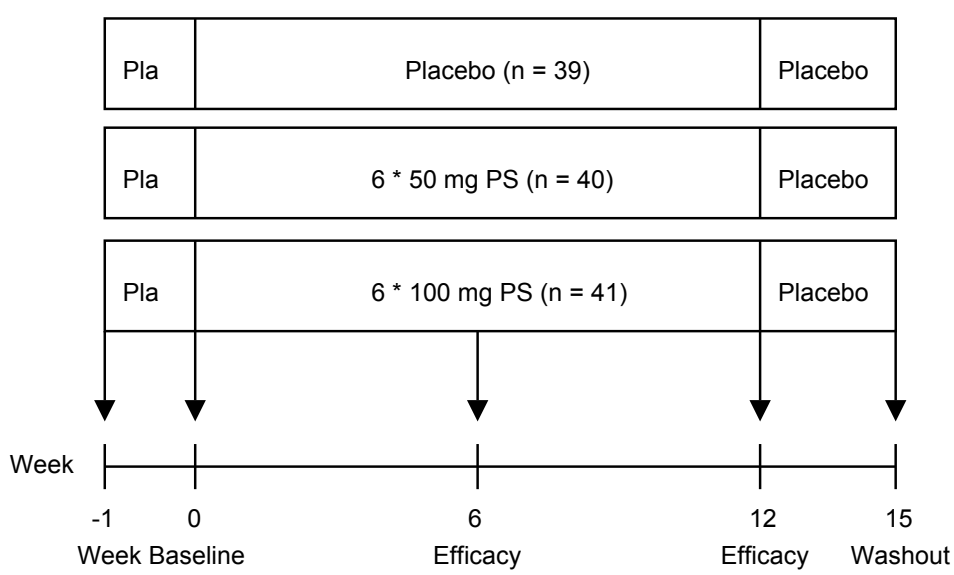
* AACD stands for Age-Associated Cognitive Decline.

Study Design

The study was conducted according to a randomized, double-blind, placebo-controlled, parallel group design. Subjects were consecutively assigned to the placebo, the 300 mg S-PS, or the 600 mg S-PS group following a predetermined order based on a randomization schedule using balanced blocks of 6 subjects. After psychological and medical screening, subjects underwent a training session followed by a 1-week placebo lead-in, 12 weeks of

treatment (placebo, 300 mg or 600 mg S-PS), and a 3-week placebo washout (see figure I). The neuropsychological test assessments took place at baseline, after 6 weeks and 12 weeks of treatment, and after washout.

Figure I. Treatment Schedule of the study to the influence of a 12-weeks treatment with two dosages phosphatidylserine (PS) or placebo on cognition in subjects with Age-Associated Memory Impairment.



Treatment

Phosphatidylserine Leci-PS-40P, a substance of food-grade quality (supplied by Novartis Consumer Health SA, Nyon, Switzerland), is produced from soya lecithin by enzymatic transesterification. The product Leci-PS-40P is a powder and contains 40% PS, 13% phosphatidylcholine, 9% phosphatidylethanolamine, 5% phosphatidylinositol, 5% phosphatidic acid, and 28% polyunsaturated fatty acids.

The study substance was packaged in 7-day packs containing 3 blister trays, each containing 14 soft gelatin capsules. Each active capsule contained a phospholipid mixture of the composition described above in an amount equivalent to 0 mg (placebo), 50 mg or 100 mg pure S-PS. The mixture was diluted with medium chain triglycerides (MCT) oil to fill the remainder of the soft gelatin capsule. The MCT oil contained 95% polar lipids (coconut and palm oil) and 5% carbohydrates. Two capsules were taken three times daily: two at breakfast, two at lunch, and two at dinner. S-PS 50 mg, S-PS 100 mg, and placebo capsules looked and tasted the same.

Outcome measures

Visual Verbal Learning Test (VVLT).

This test is an improved version of one originally devised by Rey (Brand & Jolles, 1985; Rey, 1964). During this test 15 monosyllabic words are presented for 1 second on a computer monitor. There is a 2-second inter-stimulus interval between words. After word presentation the subjects are requested to report in any order as many words they can remember (immediate recall). This procedure is repeated five times. The total immediate recall is the sum of the immediate recall measured in the 5 different trials. After 20 minutes, delayed recall and

delayed recognition are tested. During the delayed recognition test a list of 30 words is presented with 15 words from the learned list and 15 new (but comparable) words. Each word is presented for 1 second, and the maximum time interval between two words is 3 seconds. The subjects are requested to push one of two hand-held buttons: green for yes with the preferred hand if they recognize the word, and red for no with the other hand. The number of correctly recognized words, corrected for the subjects' response tendency (sensitivity measure A'), is determined by means of the following formula: A'=1-1/4(FR/CR+(1-CR)/(1-FR)). CR is the number of correctly recognized words (hits) and FR is the number of falsely recognized words (misses) (Pollack & Norman, 1964). A' was arcsin transformed before it was used in statistical analyses because of its skewed distribution. Delayed recognition reaction time was also measured.

Memory Scanning test.

Memory scanning measures the speed of the memory search process. The underlying principle is that the extra time needed to complete a test in which there is a stepwise increase in the amount of information to be kept in memory, is a measure of the ease with which information is processed in working memory (Sternberg, 1975). Briefly, 20 items are to be crossed out on four test sheets containing matrices of letters in 10 lines by 12 columns. The items to be crossed out have to be memorized before the subtask is started. In the first subtask, the memory set contains one item, in the second subtask two items, and so on. With increasing memory load, each task invariably takes more time to complete. Individual slopes, intercepts, and linearities of the completion time are calculated as a function of memory load as measures of the speed of processing information in working memory. Memory scanning slopes and intercepts were used in this study.

Verbal Fluency test.

The fluency test can be regarded as a measure of strategy-driven retrieval of information from semantic memory (Luteijn & van der Ploeg, 1983). The subjects are asked to produce, within 1 minute, as many four-letter words as possible starting with a given letter. The number of correct responses and errors are recorded. Nonsense words are not accepted, but names, conjugations and plurals are allowed. Starting letters were H, L, R and M. These letters yielded a similarly high number of correct responses (average: 9.5 to 10.7 words) in a population aged between 58 and 83 years with an average age of 70 (Houx, unpublished data).

Stroop Color Word Test (SCWT).

The SCWT has often been used to test selective attention (Houx et al., 1993). The test involves three cards each displaying a hundred stimuli: color names, colored patches, and color names printed in incongruously colored ink. The amount of extra time needed to discard irrelevant but very salient information (verbal) in favor of a less obvious aspect (color naming) is recorded. The outcome parameters of this test are the time needed to complete each subtest. The interference denotes the percentage of extra time needed to complete card III, relative to the average of cards I and II: (time card III / ((time card I + time card II)/2)) * 100%.

Signal Detection Test (SDT).

The SDT measures the ability to continuously scan the entire computer screen, searching for rarely occurring 'signals' (Schuhfried, 1991). During the task, 20 dots are presented on the screen in a random fashion. Every second three dots change position. When four dots form a square, the subject has to push a button as quickly as possible (within 2 seconds). In this

study the perceptual sensitivity measure A' and the reaction time were taken as dependent variables.

Motor Choice Reaction Time (MCRT).

Speed of information processing was assessed by measuring reaction times (RTs) as a function of task complexity (Houx & Jolles, 1993). The test consists of a simple, choice and incompatible reaction time test. This yields RTs consisting of an initiation phase (time from stimulus onset until release of a hold button) and a movement phase (time from release of the hold button until the response button is pushed). The measures used for analysis are the median initiation RTs of the simple, the choice, and the incompatible condition.

Concept Shifting Test (CST).

The CST consists of three parts (Jolles et al., 1995). On each test sheet, 16 small circles (diameter=15 mm) are grouped in a larger circle, with a radius of 8 centimeters. In the smaller circles, the test items (numbers (A), letters (B), or both (C)) appear in a fixed random order. Subjects are requested to cross out the items in the right order. In parts A and B, the subjects have to connect the numbers (1-2-3-etc.) and the letters (A-B-C-etc.) respectively. In part C, the subject is requested to alternate between these sequences (1-A-2-B-etc.). An exact estimate of the slowing due to shifting between concepts can be obtained by calculating the concept shifting interference, by comparing part C (digits and letters) with part A and part B.

Tower of London (TOL).

The TOL is a test of planning (Shallice, 1982). The test consists of three colored balls, which must be arranged on three sticks to match a picture with the goal positions. Varying the minimum number of moves to reach the goal positions alters the complexity of the problem. Each trial consists of a 2-, 3-, 4-, 5-, 6- and 7-move problem. Prior to each problem the subjects are told the minimum number of moves in which the problem can be solved. The number of moves, time to solve the problem, and time between presentation of the goal positions and the first move (decision time) are recorded. In this study the TOL was only assessed twice: at baseline and after 12 weeks of treatment. The maximum number of steps was used in this study.

Primary and secondary outcome measures.

The Visual Verbal Learning Test provided the primary outcome measures, namely, delayed recall, delayed recognition reaction time, and delayed recognition sensitivity. These three primary variables give an indication of long-term memory performance. Total immediate recall of the verbal learning test, slope and intercept of the memory scanning, fluency, stroop interference, sensitivity and reaction time of the signal detection test, reaction times of the MCRT (simple, choice and incompatible condition), the concept shifting interference, and the maximum number of steps of the TOL were the secondary outcome measures.

Statistical analyses

Data collected at the end of the placebo lead-in were taken as baseline scores, and data collected after 6 and 12 weeks of treatment were taken as the outcome measures. The MANOVA procedure was used to analyze the data in SPSS 8.0 for Windows. For the outcome measures collected after 6 weeks of treatment and at the end of the active treatment period (week 12), its corresponding baseline scores were entered as covariates in a two-way 3x2 analysis of variance, using treatment (0, 300, 600 mg) and the 'severity of memory decline' (mild versus moderate) as between-subjects factor and time as within-subjects factor

in a repeated measures design. All outcome measures were analyzed separately and possible significant differences were corrected with Bonferroni-Holme correction for multiple hypothesis testing (Holm, 1979). Similar analyses were carried out to evaluate changes during the wash-out period. The outcome scores assessed at week 15 were analyzed in a MANOVA with the baseline scores as covariate.

Results

Primary Outcome variables

The means and standard errors of the primary outcome variables and the secondary outcome variables are listed in table III. There were no effects of treatment on long-term memory performance for delayed recall ($F_{2,113}$=1.25, ns), delayed recognition sensitivity ($F_{2,113}$=0.15, ns) and delayed recognition reaction time ($F_{2,113}$=1.33, ns) after 6 and 12 weeks of S-PS treatment (see figure II for delayed recall). There was a significant effect of 'severity of memory decline' for delayed recall ($F_{1,113}$=23.29, p<0.001) and delayed recognition sensitivity ($F_{1,113}$=17.64, p<0.001). Subjects with moderate memory decline performed lower on delayed recall and delayed recognition sensitivity compared with that of subjects with mild memory decline. There was no 'severity of memory decline' effect for delayed recognition reaction time ($F_{1,113}$=0.15, ns).

After the wash-out period there were no treatment effects for delayed recall ($F_{2,113}$=0.71, ns), delayed recognition sensitivity ($F_{2,113}$=0.95, ns) and delayed recognition reaction time ($F_{2,113}$=0.80, ns). There were no 'severity of memory decline' effects for delayed recall ($F_{1,113}$=0.61, ns), delayed recognition sensitivity ($F_{1,113}$=8.52, ns after Bonferroni-Holme correction with p=0.004) and delayed recognition reaction time ($F_{1,113}$=0.51, ns) after the wash-out-periods.

None of the MANOVA results for the primary outcome variables showed interaction effects between treatment and 'severity of memory decline', or between treatment and time.

Secondary outcome variables

The MANOVA analysis of the secondary outcome measures revealed the same pattern of results as the primary outcome measures did. There were no effects of treatment at week 6 and week 12 for total immediate recall ($F_{2,113}$=0.54, ns), memory scanning intercept ($F_{2,113}$=1.28, ns), memory scanning slope ($F_{2,113}$=2.82, ns), fluency ($F_{2,113}$=0.48, ns), stroop interference ($F_{2,113}$=1.40, ns), signal detection sensitivity ($F_{2,113}$=1.77, ns), signal detection reaction time ($F_{2,113}$=1.82, ns), MCRT simple reaction time ($F_{2,113}$=0.40, ns), MCRT choice reaction time ($F_{2,113}$=0.87, ns), MCRT incompatible reaction time ($F_{2,113}$=0.85, ns) and for concept shifting interference ($F_{2,113}$=3.32, ns after Bonferroni-Holme correction with p=0.040). There was also no treatment effect for the TOL maximum number of steps ($F_{2,113}$=1.38, ns) after week 12. 'Severity of memory decline' effects on total immediate recall were present. Immediate recall performance was worse in the moderate subgroup than in the mild subgroup ($F_{1,113}$=19.84, p<0.001). The other secondary variables did not show 'severity of memory decline' effects after 6 and 12 weeks of treatment.

No significant treatment differences were measured after the washout period for total immediate recall ($F_{2,113}$=0.29, ns), memory scanning intercept ($F_{2,113}$=1.15, ns), memory scanning slope ($F_{2,113}$=1.03, ns), fluency ($F_{2,113}$=2.77, ns), stroop interference ($F_{2,113}$=0.20, ns), signal detection sensitivity ($F_{2,113}$=0.06, ns), signal detection reaction time ($F_{2,113}$=1.08, ns),

Table IIIa. Means (± SE) of the primary and secondary outcome variables for the treatment groups at baseline, at 6 and 12 weeks after treatment (wk 6 and wk 12), and after a wash-out period of 3 weeks (wk 15). The number of subjects (n) is 39 in the placebo group, 40 in the 300 mg PS group and 41 in the 600 mg PS group.

Primary Outcome Variable	**Treatment Group**	**Mn (se) Wk 0 Baseline**	**Mn (se) Wk 6 Efficacy**	**Mn (se) Wk 12 Efficacy**	**Mn (se) Wk 15 Washout**
Delayed recall (# words)	Placebo	8.4 (0.4)	9.5 (0.4)*	10.3 (0.5)*	9.3 (0.5)
	300 mg PS	7.7 (0.6)	8.8 (0.5)*	8.6 (0.6)*	8.3 (0.50)
	600 mg PS	8.2 (0.5)	8.8 (0.5)*	9.4 (0.5)*	9.3 (0.5)
Recognition Sensitivity (%)	Placebo	95.4 (0.7)	95.8 (0.6)*	96.7 (0.4)*	97 (0.4)
	300 mg PS	93.7 (0.8)	95.1 (0.7)*	95 (0.8)*	94.2 (1.1)
	600 mg PS	94.6 (0.7)	95.6 (0.5)*	95.7 (0.6)*	95.4 (0.8)
Recognition RT (msec)	Placebo	770.2 (18.5)	729.7 (15.5)	714.8 (15.4)	713.6 (13.6)
	300 mg PS	764.8 (16.9)	732.1 (13)	740.5 (13.1)	722.0 (13.5)
	600 mg PS	788.7 (20)	758.6 (16.7)	745.9 (14.7)	739.8 (16.5)
Secondary Out-come Variables	**Treatment Group**	**Mn (se) Wk 0 Baseline**	**Mn (se) Wk 6 Efficacy**	**Mn (se) Wk 12 Efficacy**	**Mn (se) Wk 15 Washout**
Immediate Total Recall (# words)	Placebo	44.7 (1.5)	47.5 (1.3)*	49.9 (1.4)*	49.2 (1.4)
	300 mg PS	41.2 (1.5)	45 (1.3)*	44.4 (1.7)*	45.4 (1.5)
	600 mg PS	44.2 (1.6)	46.3 (1.6)*	47.3 (1.2)*	48.2 (1.3)
Memscan Slope	Placebo	13.3 (0.8)	12.9 (0.8)	13.9 (0. 8)	14.1 (1)
	300 mg PS	13.8 (1.1)	14.2 (0.9)	15.7 (1.2)	14.7 (1)
	600 mg PS	14.4 (1)	14 (0.8)	13.8 (0.8)	14.4 (0.8)
Memscan Intercept (sec)	Placebo	27.3 (0.9)	27.6 (0.9)	27.7 (0.7)	27.7 (1)
	300 mg PS	30.7 (1)	32 (1.3)	33.6 (1.5)	32.6 (1.3)
	600 mg PS	30.5 (1.1)	31 (1.1)	32 (1.2)	32.4 (1.3)
Fluency (# words)	Placebo	11.5 (0.8)	12.3 (0.7)	12.1 (0.7)	13 (0,7)
	300 mg PS	9.8 (0.5)	10.6 (0.7)	11.2 (0.6)	10.8 (0.8)
	600 mg PS	10.7 (0.6)	11.1 (0.6)	10.9 (0.7)	12.2 (0.6)
Stroop Interference (sec)	Placebo	83.8 (4.4)	77.8 (3.7)	78.4 (3.8)	73.1 (3.3)
	300 mg PS	87.5 (4.9)	83.2 (4.4)	76.4 (4.5)	74.2 (4.1)
	600 mg PS	92.3 (4.8)	91.3 (5.1)	86.1 (3.6)	82.3 (3.6)
SDT Sensitivity (%)	Placebo	0.7 (0.02)	0.7 (0.02)	0.7 (0.02)	0.7 (0.02)
	300 mg PS	0.7 (0.01)	0.7 (0.02)	0.7 (0.01)	0.7 (0.02)
	600 mg PS	0.6 (0.02)	0.7 (0.02)	0.7 (0.02)	0.7 (0.02)
SDT RT (msec)	Placebo	843.3 (14.7)	855.4 (12.8)	830.5 (14.2)	842.7 (16.4)
	300 mg PS	877.5 (15.8)	892.2 (14.7)	880.8 (15.1)	873.4 (22.4)
	600 mg PS	946.0 (17.4)	945.2 (22.2)	889.9 (14.7)	913.8 (20.5)

* denotes a significant 'severity of memory' effect, which indicates a difference in performance between subjects with moderate memory decline and subjects with mild memory decline.

Table IIIb. Continued. Means (± SE) of the secondary outcome variables for the treatment groups at baseline, at 6 and 12 weeks after treatment (wk 6 and wk 12), and after a wash-out period of 3 weeks (wk 15).

Secondary Outcome Variables	Treatment Group	Mn (se) Wk 0 Baseline	Mn (se) Wk 6 Efficacy	Mn (se) Wk 12 Efficacy	Mn (se) Wk 15 Washout
MCRT Simple RT (msec)	Placebo	307.5 (5.5)	310.1 (5.9)	312.6 (6.3)	308.4 (4.9)
	300 mg PS	325.5 (6.3)	323.1 (7.7)	320.4 (7.9)	318.3 (7.1)
	600 mg PS	333.7 (7.5)	329.3 (7.9)	328.2 (7.6)	328.2 (7.5)
MCRT Choice RT (msec)	Placebo	54.7 (4.4)	51.4 (4.5)	51.7 (5.7)	56.8 (4.3)
	300 mg PS	38.6 (3.9)	48.7 (3.7)	43.7 (5.3)	53. 6 (3.8)
	600 mg PS	54.7 (4.8)	53.7 (5.4)	62.1 (4. 6)	55.7 (4.3)
MCRT Incomp RT (msec)	Placebo	128.8 (7)	126 (7.2)	114.4 (6.6)	107 (5.4)
	300 mg PS	129.4 (7.7)	117.7 (7.4)	113.8 (7.7)	96.9 (7.8)
	600 mg PS	144.5 (8.8)	132.3 (7.1)	127.2 (6.9)	127 (6.3)
CST Interference (sec)	Placebo	52.44 (6.1)	47.9 (5)	35.9 (3.9)	44.6 (4.3)
	300 mg PS	51.70 (7.2)	55.3 (6)	48.1 (4.9)	46.9 (5.4)
	600 mg PS	57.34 (6.4)	61.2 (6.1)	55.8 (5)	55.8 (6.2)
Tower of London	Placebo	6.82 (0.1)	Not assessed	6.7 (0.1)	Not assessed
	300 mg PS	6.53 (0.2)		6.6 (0.2)	
	600 mg PS	6.80 (0.1)		6.9 (0.1)	

MCRT simple reaction time ($F_{2,113}$=1.53, ns), MCRT choice reaction time ($F_{2,113}$=0.95, ns), MCRT incompatible reaction time ($F_{2,113}$=3.74, ns after Bonferroni-Holme correction with p=0.027)and for concept shifting interference ($F_{2,113}$=0.14, ns). There were no 'severity of memory decline' effects after the washout period. Furthermore there were no significant interactions between treatment and 'severity of memory decline' or between treatment and time at week 6, week 12 or after the wash-out period.

Discussion

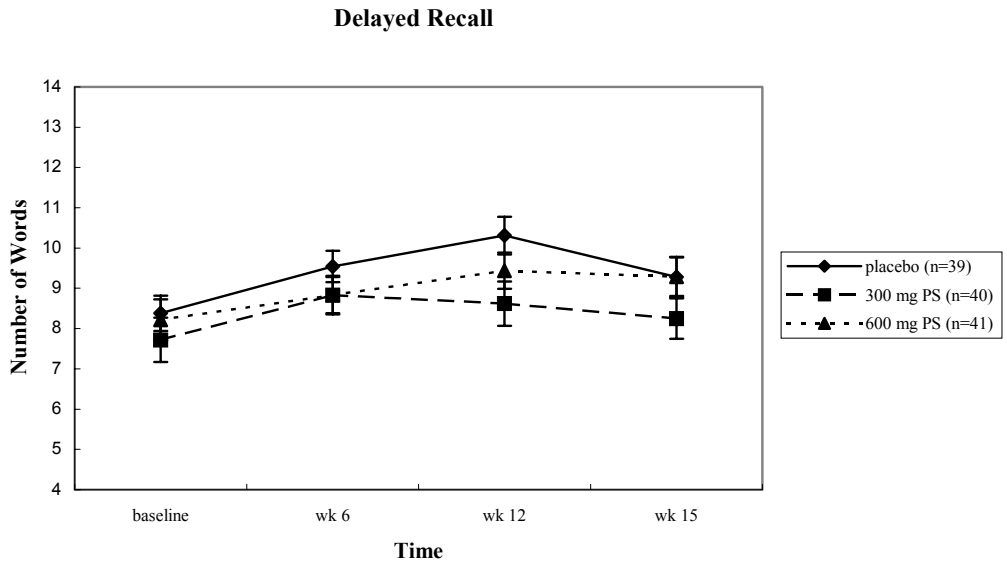
This double-blind, placebo-controlled study showed that S-PS treatment did not have any effect on cognitive performance in subjects older than 57 years of age with age-associated memory impairment.

We will first discuss some methodological issues that might have influenced the results of this study (for example due to sampling error). There might have been a confounding factor present in the distribution of the subjects between treatment groups. Although there were no significant differences in IQ, age, or sex, mean IQ appeared higher in the placebo group (mean IQ = 120) than in the treatment groups (mean IQ = 115 for 300 mg S-PS and 118 for 600 mg PS). However, none of the outcome measures at baseline, with the exception of the Concept Shifting Test and the Fluency Test scores, were significantly associated with IQ scores. Even if there was a significant group difference, the baseline correction which we applied in our analyses should have dealt with this problem.

Another factor that could have influenced the results concerns the sensitivity of the cognitive tests to detect treatment effects, defined as the test-retest reliability. The latter has been identified as the most appropriate single summary measure of reliability (Parrott, 1991). Av-

erage intraclass correlation coefficients, which are overall correlations of multiple assessments, were analyzed over five assessments (including training). The analyses revealed an adequate test-retest reliability higher than 0.8 for the primary variables to detect treatment effects. The test-retest reliabilities was higher than 0.8 for the secondary variables, except for choice reaction time (0.7), concept shifting interference (0.6), and the number of steps of the tower of London (0.3) which was only assessed at baseline and at week 12. This indicates that only the concept shifting test and the tower of London test may not have been sensitive enough to detect a treatment effect.

Figure II. Mean (± SE) delayed recall scores of the VVLT at baseline, at 6 and 12 weeks after treatment (wk 6 and wk 12), and after a wash-out period of 3 weeks (wk 15). The number of subjects (n) is 39 in the placebo group, 40 in the 300 mg PS group and 41 in the 600 mg PS group.



Concerning the sample size, a priori power analysis showed that 117 subjects was the minimum total sample size in a three group design, in order to detect a treatment difference on delayed recall of 1.25 (with standard deviation of 2) with a power of 80%. In case of a smaller effect size, indeed, power would have been too low. However, a smaller effect then should have emerged as a trend in the data.

A potentially important treatment-related issue is the presence of small amounts of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) in the phosphatidylserine substance used in our study. It would have been better had S-PS been the only phospholipid in the capsules because the other phospholipids may have influenced the treatment effect of S-PS. In rat studies it has been shown that the administration of PS liposomes increases calcium-dependent acetylcholine release from the cerebral cortex in anaesthetized rats, with PE being about half as active as PS whereas PC was inactive in this respect (Casamenti et al., 1979). Despite these less pronounced effects of PC and PE on acetylcholine release, the mechanisms of action of the phospholipids may have interacted with those of the S-PS treatment in our study. Eagger and colleagues (Eagger et al., 1991) showed that 150 mg tacrine (a cholinesterase inhibitor) in combination with 10.8 g lecithin (containing 15% PC) also improved cognitive function in Alzheimer patients, although the authors mentioned that lecithin was unlikely to have an influence because the treatment had

no effect on plasma choline concentrations. The lecithin dose was about 30 times higher than the effective dose of BC-PS. Therefore, although in principle we cannot exclude effects of PC and PE, we doubt whether they would have had an effect because they were present in very small amounts.
We assumed that the AAMI criteria are suitable to demonstrate a treatment effect in normally aging individuals. Crook et al. (Crook et al., 1986) published the diagnostic criteria for AAMI to stimulate research into the epidemiological, clinical characterization, and treatment aspects of 'normal' later-life memory loss. Several drugs have been tested for their cognition-enhancing effects on AAMI or age-associated cognitive decline (AACD), although no cognition enhancer has reliably and repeatedly been demonstrated to be effective (Riedel & Jolles, 1996). Subjects with AAMI may not be suitable as a clinical population in studies of drugs or nutrients. However, as most nutritional supplements are aimed at normally aging subjects, this argument may not hold for nutrient studies. AAMI-subjects hardly differ from 'normal-aging' subjects and for this purpose, application of the AAMI criteria may be a well-tuned pragmatic approach in efficacy studies of nutritional supplements for the elderly. As such, the AAMI criteria fulfill the function of excluding successfully aging subjects from study participation. Smith and colleagues (Smith et al., 1991) discussed some problems of reliability and expressed concerns regarding the AAMI criteria. One of their suggestions was the use of age-appropriate norms, since the AAMI criteria do not consider the discontinuity of normal test performance between the younger old and the older old. We used the AAMI criteria so that we could compare our results with those of previous PS studies (Crook, 1998; Crook et al., 1991; Gindin et al., 1993). We also used age-appropriate norms to define the group with more severe memory decline.

Finally, we assumed that ingested S-PS would be available in the brain. Whereas nothing is known about the amount of BC-PS or S-PS that passes through the gastrointestinal tract and the blood-brain barrier after oral administration in humans. In rats, orally administered radioactive-labeled BC-PS (^{14}C-PS) passes the gastrointestinal tract very slowly, and an intravenously injected dose was halved very rapidly and only 0.25% of an injected dose reached the brain tissue after 20 minutes (Orlando et al., 1987). However in humans, Rosadini and colleagues (Rosadini et al., 1990) using indirect quantitative EEG methods, showed an increase of the power on the 'alpha' frequency at the anterior electrode after a 50-mg dose of intravenously administered BC-PS, and over the whole scalp after a 75-mg dose of BC-PS. This implies that BC-PS induced cerebral activity after intravenous administration. Plasma concentrations of PS after oral administration in humans have not been reported in the literature.

In future studies, attention should be paid to the fatty-acid content of PS. Essential fatty acids may control the modulation of neuronal membrane fluidity and thus might influence cognitive functions (Yehuda et al., 1999). Treatment with a 1:4 ratio of n-3 and n-6 fatty acids improved mood, cooperation, appetite, sleep, ability to navigate in the home, and short-term memory in a placebo-controlled trial with 100 Alzheimer patients (Yehuda et al., 1996). In our study, linoleic acid (n-6) accounted for approximately 58% of the polyunsaturated fatty acids in and linolenic acid (n-3) for 7%. No information is available of the fatty acid content of PS used in other human studies. In animal studies (Blokland et al., 1999; Sakai et al., 1996), there was a large difference in fatty acid content of the S-PS and BC-PS formulas used, and

even between the different S-PS formulas. This might be important for the efficacy of specific PS formulas.
Another aspect that needs to be addressed is the way of production of S-PS by enzymatic conversion. Some enzyme may be included in the final product, which may lead to a partial degradation of S-PS with time. In our study, the capsules contained 50% of the initial value of S-PS after 15 months. This final level was still higher than the level of supplementation used in studies with BC-PS, and the last treatment was finished 11 months after the capsules were prepared.

Despite these reservations, PS would appear to have only doubtful cognitive enhancing effects in subjects with AAMI. PS did not influence cognitive functions in our AAMI population, whereas Crook and colleagues showed that S-PS and BC-PS enhanced both name recall immediately and an hour after introduction, and learning and recall of written information in AAMI subjects (Crook, 1998). However, this effect was only present in the subgroup of patients with the most severe cognitive impairment. Crook compared the placebo and BC-PS group from the 1991 study (Crook et al., 1991) with a new S-PS group, without introducing a new placebo group. Thus the data were not analyzed according to a double-blind procedure, which is very important in clinical trials. Gindin and colleagues showed, in a double-blind, placebo-controlled study, a significant improvement of memory and mood in 72 AAMI-subjects (Gindin et al., 1993). In contrast to the study by Crook et al., subjects with higher baseline scores had an improved memory function after treatment.
The behavioral effects of S-PS have also been studied in animals. Blokland and colleagues (Blokland et al., 1999) compared the behavioral effects of an intraperitoneal injection of BC-PS with S-PS and E-PS in a placebo controlled rat study. BC-PS and S-PS had similar effects on tests of avoidance learning, but not on tests of spatial discrimination learning. Sakai and colleagues (Sakai et al., 1996) also found that orally administered S-PS and BC-PS improved scopolamine-induced deterioration of passive avoidance. Compared to the cognition-enhancing effects in rats of other cholinesterase inhibitors, such as metrifonate (Blokland et al., 1995; van der Staay et al., 1996), the magnitude of the effects obtained with BC-PS and S-PS were only marginal.
Thus while BC-PS and S-PS may have cognition-enhancing effects in animals, their effects in humans are not yet clear. This difference in effect might be caused by the difference between animal and human research. In animal research the methodological conditions can be controlled more accurately. For instance, the diet of the animals is the same, whereas in human PS studies, including our study, the diet of the free-living volunteers was not controlled. The diet composition, for example, a high fat versus a low fat diet, might influence the uptake of S-PS in the gastrointestinal tract.

In summary, this study showed that oral administration of S-PS did not improve memory or other cognitive functions in people suffering from AAMI. Since others (Crook et al., 1991; Gindin et al., 1993) have shown cognition-enhancing effects of BC-PS in different subgroups of AAMI subjects, and the plasma concentration of PS after oral administration has not been measured in humans, the efficacy of PS is still questionable. Future research could benefit from the development of methods to assess changes in the relatively low plasma concentrations of PS after its oral administration in humans.

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5

Safety of Soy-derived Phosphatidylserine in Elderly People

Phosphatidylserine (PS) is a phospholipid which has been claimed to enhance neuronal membrane function. The phospholipid can be derived from several sources. Earlier animal and human studies used brain cortex derived PS (BC-PS), of which the human tolerability of 300 mg daily in 130 patients has been studied by Cenacchi and colleagues (Cenacchi et al., 1987). The human tolerability of PS derived from soy-bean (S-PS) has not been reported until now, although it is widely sold as a nutritional supplement which may improve cognitive function in the elderly. We report the results of a study in which the safety of two dosages of S-PS (300 mg and 600 mg) is evaluated in elderly with memory complaints.

Subjects were 120 elderly (>57 years) of both sexes who fulfilled the more stringent criteria for age-associated memory impairment (AAMI); some also fulfilled the criteria for age-associated cognitive decline. Subjects were allocated at random to one of the three treatment groups: placebo, 300 mg S-PS daily, or 600 mg S-PS daily. Standard biochemical and hematological safety parameters, blood pressure and heart rate were assessed at baseline, and after 6 and 12 weeks of treatment.

No significant differences were found in any of the outcome variables between the treatment groups after Bonferonni-Holme correction.

In conclusion, soy derived phosphatidylserine (S-PS) is a safe nutritional supplement for older persons if taken up to a dosage of 200 mg three times daily.

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Introduction

Phosphatidylserine (PS) is a phospholipid which is claimed to improve memory performance in subjects suffering from age-associated memory impairment (AAMI) (Crook et al., 1991) or Alzheimer's Disease (Crook et al., 1992). PS can be derived from several sources. Earlier animal and human studies used brain cortex derived PS (BC-PS). PS derived from soy-bean (S-PS) and egg sources (E-PS) have more recently been developed. S-PS, but not E-PS, had similar effects as BC-PS on tests of avoidance learning in rats (Blokland et al., 1999).
The human tolerability of oral BC-PS 300 mg daily in 130 patients has been studied by Cenacchi and colleagues (Cenacchi et al., 1987). BC-PS induced significant reductions in uric acid and serum glutamate pyruvate transaminase (SGPT) in male, although these results were of negligible clinical relevance. Studies in animals have also shown that BC-PS was without significant toxicity (Heywood et al., 1987).
The biochemical and hematological tolerability of S-PS in humans, which is widely sold as a nutritional supplement, has not been reported until now.
The present study is the first in which the safety of two dosages of S-PS (300 mg and 600 mg) is evaluated in a double-blind placebo-controlled study of healthy subjects with AAMI. The specific objectives of this study were to evaluate the safety of these two daily dosages of S-PS after 6 and 12 weeks of treatment, compared to placebo. The relevant safety parameters were derived from biochemical and hematological variables and the examination of vital signs and adverse events.
The cognitive effects of S-PS have also been studied in this population and have been reported elsewhere (Jorissen et al., 2001). Briefly, that study showed no change in cognitive performance after 12 weeks of daily treatment with phosphatidylserine 300 mg or 600 mg (Jorissen et al., 2001).

Methods

Subjects
Subjects were recruited through advertisements in the local newspaper and on local television, and through posters in general practitioners' waiting rooms and at places where the elderly meet, such as sport or recreation centers for the elderly. All subjects were older than 57 years and fulfilled the criteria for Age Associated Memory Impairment; AAMI (Crook et al., 1986). AAMI is characterized by complaints of memory impairment in every day life, memory test performance at least one standard deviation below the mean established for young adults on at least one out of three memory tests, adequate intellectual function, and absence of dementia or depression. Further exclusion criteria were: evidence of delirium, confusion, or other disturbances of consciousness; any neurological disorder that could produce cognitive deterioration as determined by medical history, clinical neurological examination, or neuroradiological examination; history of any infective or inflammatory brain disease; evidence of significant cerebral vascular pathology as determined by a Hachinski Ischemic Score (HIS) over 4; history of head injury; current psychiatric diagnosis according to DSM-IV criteria of a major psychiatric disorder; current diagnosis or history of alcoholism or drug dependence; any medical disorder that could produce cognitive deterioration; use of any psychotropic drug or any other drug that may significantly affect cognitive function during the month prior to psychometric testing; known hypersensitivity of PS.

The study was approved by the Medical Ethics Committee of the University Hospital of Maastricht and all subjects gave written informed consent.
After the screening procedure 132 subjects entered the study and 120 subjects completed the treatment (placebo: n=39, 300 mg S-PS: n=40 and 600 mg S-PS: n=41). Drop-outs were distributed equally over the three groups: 4 in the placebo group, 5 in the 300 mg S-PS group and 3 in the 600 mg S-PS group. The reasons for drop-out were unrelated to the treatment.

Study Design
The study was conducted according to a randomized, double-blind, placebo-controlled, parallel group design. Subjects were consecutively assigned to the placebo, the 300 mg S-PS, or the 600 mg S-PS group following a predetermined order based on a randomization schedule using balanced blocks of 6 subjects. After psychological and medical screening, subjects underwent a training session followed by a 1-week placebo lead-in, 12 weeks of treatment (placebo, 300 mg or 600 mg S-PS), and a 3-week placebo washout. The biochemical and hematological examination took place at baseline, and after 6 weeks and 12 weeks of treatment.

Treatment
Phosphatidylserine Leci-PS-40P, a substance of food-grade quality, is produced from soya lecithin by enzymatic transesterification. The product Leci-PS-40P is a powder and contains 40% PS, 13% phosphatidylcholine, 9% phosphatidyl-ethanolamine, 5% phosphatidylinositol, 5% phosphatidic acid, and 28% polyunsaturated fatty acids.
The study substance was packaged in 7-day packs containing 3 blister trays, each containing 14 soft gelatin capsules. Each active capsule contained a phospholipid mixture of the composition described above in an amount equivalent to either 0 mg (placebo), 50 mg or 100 mg pure S-PS. The mixture was diluted with medium chain triglycerides (MCT) oil to fill the remainder of the soft gelatin capsule. The MCT oil contained 95% polar lipids (coconut and palm oil) and 5% carbohydrates. Two capsules were taken three times daily: two at breakfast, two at lunch, and two at dinner. S-PS 50 mg, S-PS 100 mg, and placebo capsules were indistinguishable on the basis of appearance and taste.

Medical Assessments
Biochemical safety parameters consisted of alanineaminotransferase (ALAT), aspartaataminotransferase (ASAT), Alkaline Phosphatase, Calcium, Glucose, Potassium, Creatinine, Sodium, Urea, Uric Acid and Bilirubine. Hematological safety parameters consisted of white blood count, lymphocytes, monocytes, neutrophils, platelet count, basophiles, eosinophiles, red blood count, hematocrit and hemoglobin. Safety parameters derived from urinalysis consisted of protein, glucose and blood. The samples were analyzed by the hematology-oncology department and the clinical chemistry department of the Academic Hospital Maastricht. All these parameters were assessed at baseline, after 6 weeks and after 12 weeks of treatment. Resting diastolic pressure, systolic pressure and heart rate were measured, using an automatic recording device (Critikon Dinamap®8100).

Subjective Side Effects
The subjects were asked to fill out a four point rating scale, which dealt with 27 adverse events. Each item consisted of four categories: seldom or never (1), sometimes (2), regularly

(3) and often (4). The rating scale described the following 27 side effects: headache, falling asleep, restlessness, tense feeling on chest, problems with digestion, acidity of the stomach, intolerance to light, troubled concentration, feels short of breath / dyspnea, tires easily during daytime, diarrhea, feels faint, heart palpitations, intolerance to noise, dry mouth, dizziness, increased appetite, nausea, transpiration, cries more easily, decreased libido, irritable, loss of initiative, awake at night, itch, flatulence, decreased appetite. Due to the generally low frequencies of individual items, a sum score of all side effect ratings served as a global measure of 'summed side effects'.

Statistical Analysis

SPSS 9.0 was used for the MANOVA analyses. For the outcome measures collected at week 6 and week 12, their corresponding baseline scores were entered as covariate in a 3x2 analysis of variance, using treatment (0, 300, 600 mg) and gender as between-subjects factor and time as within-subjects factor in a repeated measures design. Each safety parameter was analyzed separately. All a priori significant group differences were corrected for multiple hypothesis testing with the Bonferroni-Holme method. According to this rule, the lowest p-value has to be lower than 0.05 divided by the number of hypotheses tested (h), the second p-value has to be lower than 0.05/(h-1), the third p-value has to be lower than 0.05 / (h-2) etc., and the last p-value has to be lower than 0.05/1.
Since the assumption of normality which is necessary for the MANOVA analyses was violated for the side effects rating scale, the non-parametric Kruskal-Wallis test was used for testing the difference between week 12 and baseline of the summed side effects.

Results

Missing Values
There were 22 missing values out of a total of 8640 values (0.25%), due to problems with taking blood samples. In the results tables I to III the actual number of subjects (n) is added if there was a missing value for the specific parameter.

Biochemical parameters
There were no significant differences between treatment groups at week 12 for the following biochemistry blood parameters: ALAT ($F_{2,113}$=1.44, ns), ASAT ($F_{2,113}$=0.30, ns), alkaline phosphatase ($F_{2,112}$=0.82, ns), calcium ($F_{2,113}$=0.31, ns), glucose ($F_{2,109}$=0.19, ns), potassium ($F_{2,113}$=0.04, ns), creatinine ($F_{2,113}$=0.58, ns), sodium ($F_{2,113}$=0.22, ns), urea ($F_{2,113}$=0.54, ns), uric acid ($F_{2,113}$=0.81, ns) and bilirubine ($F_{2,113}$=0.08, ns). An interaction between treatment and time was present for ALAT. ALAT increased in the placebo group between week 6 and week 12 ($F_{2,114}$=4.92, p=0.009).

Table Ia. Safety blood parameters Biochemistry. Means (and standard errors) of baseline, week 6 and week 12.

	Baseline			Week 6			Week 12			Normal range
	Placebo (n=39)	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	
ALAT	24.64 (1.37)	22.05 (0.95)	23.66 (1.03)	24.38 (1.71)	23.18 (1.22)	23.90 (1.03)	27.46 (2.04)	22.50 (1.06)	22.02 (1.05)	m: 5-40 u/l f: 5-35 u/l
male	23.8 (1.45)	24.21 (1.23)	25.25 (1.55)	23.05 (1.28)	25.32 (1.85)	25.05 (1.52)	26.65 (1.90)	25.68 (1.32)	23.35 (1.61)	
female	25.53 (2.38)	21.00 (1.32)	22.14 (1.32)	25.79 (3.26)	21.24 (1.55)	22.81 (1.38)	28.32 (3.73)	19.62 (1.38)	20.76 (1.33)	
ASAT	26.15 (1.34)	23.23 (0.63)	24.39 (0.95)	25.36 (1.71)	22.80 (0.81)	23.85 (0.95)	26.33 (1.26)	23.45 (0.76)	24.15 (0.85)	m: 5-40 u/l f: 5-35 u/l
male	24.65 (1.19)	23.58 (0.96)	25.61 (1.58)	24.00 (1.11)	23.05 (1.13)	25.00 (1.42)	25.05 (1.17)	25.16 (1.04)	25.25 (1.04)	
female	27.74 (2.43)	22.90 (0.84)	23.24 (1.05)	26.79 (3.32)	22.57 (1.18)	22.76 (1.25)	27.68 (2.28)	21.90 (1.01)	23.10 (1.32)	
Alkaline Phosphatase	79.56 (3.02)	81.20 (2.79)	82.68 (3.44)	79.28 (3.42)	79.00 (2.41)	80.58 (3.29) (n=40)	79.90 (2.96)	78.95 (2.29)	80.37 (3.10)	30-125 u/l
male	83.15 (4.83)	81.90 (4.73)	79.30 (4.53)	83.55 (5.32)	79.47 (3.84)	78.58 (4.70)	83.45 (4.36)	78.37 (3.80)	78.75 (3.72)	
female	75.79 (3.48)	80.57 (3.25)	85.90 (5.17)	74.79 (4.14)	78.57 (3.09)	82.38 (4.67) (n=19)	76.16 (3.91)	79.48 (2.78)	81.90 (4.97)	
Calcium	2.43 (0.02)	2.44 (0.01)	2.42 (0.02)	2.43 (0.02)	2.43 (0.01)	2.42 (0.02)	2.43 (0.01)	2.42 (0.01)	2.42 (0.02)	2.1-2.6 mmol/l
male	2.40 (0.02)	2.42 (0.02)	2.39 (0.03)	2.41 (0.01)	2.42 (0.02)	2.40 (0.02)	2.40 (1.79)	2.44 (0.02)	2.41 (0.02)	
female	2.45 (0.02)	2.47 (0.02)	2.45 (0.02)	2.46 (0.03)	2.43 (0.02)	2.45 (0.03)	2.45 (0.02)	2.41 (0.02)	2.43 (0.02)	
Glucose	5.92 (0.18)	5.84 (0.10)	5.70 (0.14)	5.89 (0.23)	5.72 (0.09) (n=39)	5.90 (0.17)	5.85 (0.18) (n=38)	5.82 (0.15) (n=38)	5.66 (0.09)	3.1-7.8 mmol/l
male	5.83 (0.20)	5.85 (0.15)	5.75 (0.26)	5.56 (0.13)	5.75 (0.11)	6.10 (0.31)	5.79 (0.13)	6.07 (0.26) (n=18)	5.64 (0.12)	
female	6.02 (0.31)	5.83 (0.13)	5.64 (0.14)	6.24 (0.45)	5.70 (0.14) (n=20)	5.70 (0.14)	6.03 (0.35) (n=18)	5.60 (0.15)	5.67 (0.13)	
Potassium	4.37 (0.06)	4.41 (0.06)	4.44 (0.06)	4.49 (0.05)	4.52 (0.06)	4.40 (0.05)	4.30 (0.05)	4.31 (0.05)	4.40 (0.05)	3.6-5 mmol/l
male	4.39 (0.08)	4.38 (0.08)	4.39 (0.09)	4.47 (0.07)	4.48 (0.08)	4.43 (0.07)	4.29 (0.07)	4.39 (0.08)	4.45 (0.07)	
female	4.35 (0.08)	4.44 (0.08)	4.49 (0.10)	4.50 (0.07)	4.55 (0.09)	4.37 (0.07)	4.31 (0.07)	4.23 (0.07)	4.35 (0.07)	

Table I b. Continued. Safety blood parameters Biochemistry. Means (and standard errors) of baseline, week 6 and week 12.

	Baseline			Week 6			Week 12			Normal range
	Placebo (n=39)	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	
Creatinine	80.56 (2.87)	80.20 (2.34)	78.15 (2.12)	80.85 (2.56)	79.33 (2.71)	78.95 (2.03)	80.64 (2.35)	81.63 (2.63)	81.80 (2.55)	
male	92.00 (3.60)	89.63 (2.55)	87.75 (2.28)	89.50 (2.80)	91.05 (2.45)	87.60 (2.03)	91.15 (2.40)	89.26 (2.89)	93.50 (2.96)	71-110 mmol/l
female	68.53 (2.35)	71.67 (2.72)	69.00 (2.05)	71.74 (3.28)	68.71 (3.26)	70.71 (2.33)	69.58 (2.09)	74.71 (3.72)	70.67 (2.19)	
Sodium	140.74 (0.37)	140.88 (0.30)	140.51 (0.33)	141.18 (0.40)	141.05 (0.30)	140.83 (0.28)	140.85 (0.61)	140.63 (0.37)	141.27 (0.41)	
male	140.80 (0.35)	140.32 (0.57)	140.00 (0.34)	141.70 (0.47)	141.00 (0.33)	140.60 (0.41)	141.20 (1.06)	140.84 (0.67)	141.55 (0.73)	132-145 mmol/l
female	140.68 (0.68)	141.38 (0.23)	141.00 (0.55)	140.63 (0.65)	141.10 (0.50)	141.05 (0.38)	140.47 (0.58)	140.43 (0.36)	141.00 (0.39)	
Urea	5.71 (0.18)	5.70 (0.18)	5.92 (0.25)	5.71 (0.21)	6.06 (0.23)	6.12 (0.24)	6.06 (0.19)	6.08 (0.22)	6.22 (0.25)	
male	6.14 (0.22)	5.76 (0.23)	6.14 (0.36)	6.14 (0.22)	6.28 (0.25)	5.96 (0.39)	6.43 (0.29)	6.06 (0.24)	6.19 (0.38)	3-7 mmol/l
female	5.25 (0.26)	5.64 (0.28)	5.71 (0.34)	5.25 (0.33)	5.85 (0.38)	6.27 (0.29)	5.68 (0.24)	6.10 (0.37)	6.26 (0.32)	
Uric Acid	0.32 (0.01)	0.30 (0.01)	0.30 (0.01)	0.32 (0.01)	0.30 (0.01)	0.30 (0.01)	0.32 (0.01)	0.30 (0.01)	0.29 (0.01)	
male	0.36 (0.02)	0.33 (0.02)	0.34 (0.01)	0.36 (0.02)	0.34 (0.02)	0.33 (0.02)	0.36 (0.01)	0.33 (0.02)	0.33 (0.01)	m: 0.2-0.42 u/l f: 0.12-0.34 u/l
female	0.27 (0.01)	0.26 (0.01)	0.26 (0.01)	0.28 (0.01)	0.26 (0.01)	0.27 (0.01)	0.28 m(0.01)	0.27 (0.01)	0.26 (0.01)	
Bilirubine	11.00 (0.59)	11.70 (0.50)	10.59 (0.59)	10.98 (0.66)	11.05 (0.54)	10.81 (0.69)	11.15 (0.58)	11.27 (0.56)	10.35 (0.55)	
male	11.69 (0.91)	12.17 (0.64)	10.97 (0.94)	12.54 (1.04)	12.51 (0.70)	11.38 (1.06)	11.59 (1.04)	13.24 (0.69)	11.14 (0.95)	2.0-17.0 umol/l
female	10.28 (0.73)	11.26 (0.76)	10.23 (0.74)	9.34 (0.64)	9.72 (0.71)	10.28 (0.89)	10.68 (0.50)	9.49 (0.66)	9.60 (0.57)	

Table IIa. Safety blood parameters Haematology. Means (and standard errors) of baseline, week 6 and week 12.

	Baseline			Week 6			Week 12			Normal range
	Placebo (n=39)	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	
White Blood Count	7.57 (0.31)	7.07 (0.19)	7.01 (0.32)	7.30 (0.36)	6.73 (0.18) (n=39)	7.26 (0.31)	7.58 (0.29)	7.00 (0.22)	7.48 (0.32)	3.5-11 10E9/l
male	7.51 (0.33)	7.21 (0.32)	7.41 (0.49)	7.25 (0.32)	6.71 (0.27) (n=18)	7.69 (0.53)	7.75 (0.35)	7.05 (0.37)	7.87 (0.49)	
female	7.63 (0.53)	6.94 (0.21)	6.64 (0.39)	7.37 (0.69)	6.74 (0.26)	6.85 (0.32)	7.40 (0.48)	6.96 (0.27)	7.11 (0.41)	
Lymphocytes	27.03 (1.01)	27.83 (0.95)	29.22 (1.28)	29.21 (1.16) (n=38)	30.38 (1.07) (n=39)	30.83 (1.20)	29.31 (1.17)	29.10 (1.12)	30.90 (1.40)	24-50%
male	27.45 (1.58)	25.89 (1.28)	28.25 (1.83)	29.25 (1.56)	27.94 (1.49) (n=18)	30.50 (1.67)	28.35 (1.59)	27.00 (1.49)	30.55 (2.20)	
female	26.58 (1.26)	29.57 (1.29)	30.14 (1.83)	29.17 (1.77) (n=18)	32.48 (1.41)	31.14 (1.76)	30.32 (1.75)	31.00 (1.56)	31.24 (1.80)	
Monocytes	8.33 (0.35)	8.28 (0.39)	8.10 (0.30)	8.89 (0.33) (n=38)	8.59 (0.33) (n=39)	8.32 (0.28)	8.46 (0.35)	8.65 (0.32)	8.24 (0.28)	1-11%
male	9.00 (0.48)	8.74 (0.61)	8.50 (0.47)	9.45 (0.49)	9.17 (0.54) (n=18)	8.30 (0.48)	8.90 (0.38)	9.05 (0.51)	8.50 (0.38)	
female	7.63 (0.46)	7.86 (0.50)	7.71 (0.35)	8.28 (0.39) (n=18)	8.10 (0.38)	8.33 (0.31)	8.00 (0.58)	8.29 (0.40)	8.00 (0.40)	
Neutrophils	62.23 (1.17)	60.98 (1.07)	60.44 (1.33)	59.21 (1.29) (n=38)	57.82 (1.18) (n=39)	58.22 (1.15)	59.54 (1.15)	59.05 (1.29)	57.88 (1.39)	28-65 %
male	61.15 (1.79)	62.37 (1.24)	60.45 (1.80)	59.20 (1.70)	59.83 (1.65) (n=18)	58.35 (1.47)	60.15 (1.60)	61.16 (1.73)	57.85 (2.14)	
female	63.37 (1.51)	59.71 (1.69)	60.43 (1.99)	59.22 (2.03) (n=18)	56.10 (1.62)	58.10 (1.79)	58.89 (1.69)	57.14 (1.84)	57.90 (1.84)	
Platelet Count	244.49 (7.61)	242.80 (6.50)	231.39 (6.92)	242.38 (7.06) (n=38)	232.40 (6.68)	225.95 (7.04)	242.38 (7.06)	234.95 (6.28)	229.24 (7.10)	130-350 10E9/l
male	241.75 (8.36)	246.79 (10.69)	220.00 (10.18)	236.10 (8.41)	233.26 (9.67)	214.15 (9.52)	236.10 (8.41)	233.26 (9.67)	208.45 (9.24)	
female	247.37 (13.13)	239.19 (7.90)	242.24 (9.01)	249.00 (11.51) (n=18)	236.48 (8.37)	237.19 (9.93)	249.00 (11.51)	236.48 (8.37)	249.05 (8.92)	

Table IIb. Continued. Safety blood parameters Haematology. Means (and standard errors) of baseline, week 6 and week 12.

	Baseline			Week 6			Week 12			
	Placebo (n=39)	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Placebo (n=39)**	**PS 300 mg (n=40)**	**PS 600 mg (n=41)**	**Normal range**
Basophils	0.56 (0.08)	0.55 (0.08)	0.56 (0.09)	0.55 (0.09) (n=38)	0.64 (0.11) (n=39)	0.54 (0.08)	0.54 (0.08)	0.60 (0.08)	0.59 (0.08)	0-2%
male	0.45 (0.11)	0.58 (0.12)	0.55 (0.11)	0.50 (0.11)	0.39 (0.12) (n=18)	0.45 (0.11)	0.60 (0.11)	0.53 (0.12)	0.55 (0.11)	
female	0.68 (0.11)	0.52 (0.11)	0.57 (0.13)	0.61 (0.14) (n=18)	0.86 (0.16)	0.62 (0.11)	0.47 (0.12)	0.67 (0.11)	0.62 (0.11)	
Eosinophils	2.21 (0.22)	2.68 (0.28)	2.07 (0.24)	2.37 (0.25) (n=38)	2.77 (0.24) (n=39)	2.39 (0.24)	2.64 (0.27)	2.80 (0.30)	2.66 (0.29)	0-5%
male	2.05 (0.30)	2.63 (0.29)	2.50 (0.41)	2.20 (0.31)	2.72 (0.25) (n=18)	2.50 (0.41)	2.40 (0.34)	2.47 (0.22)	2.95 (0.44)	
female	2.37 (0.34)	2.71 (0.47)	1.67 (0.24)	2.56 (0.41) (n=18)	2.81 (0.40)	2.29 (0.26)	2.89 (0.43)	3.10 (0.54)	2.38 (0.36)	
Red Blood Count	4.65 (0.06)	4.69 (0.05)	4.67 (0.04)	4.65 (0.06) (n=37)	4.60 (0.05) (n=39)	4.60 (0.04)	4.73 (0.06)	4.64 (0.05)	4.62 (0.04)	m: 4.2-5.6 10E9/l f: 3.7-5.0 10E9/l
male	4.77 (0.08)	4.68 (0.06)	4.75 (0.07)	4.80 (0.07)	4.62 (0.08) (n=18)	4.69 (0.08)	4.86 (0.08)	4.67 (0.08)	4.72 (0.06)	
female	4.51 (0.08)	4.70 (0.07)	4.59 (0.05)	4.48 (0.07) (n=17)	4.58 (0.05)	4.51 (0.03)	4.60 (0.07)	4.61 (0.06)	4.52 (0.05)	
Hematocrit	0.42 (0.005)	0.42 (0.004)	0.42 (0.005)	0.42 (0.005) (n=38)	0.42 (0.005)	0.42 (0.004)	0.43 (0.005)	0.42 (0.005)	0.42 (0.004)	m: 0.4-0.52 10E9/l f: 0.36-0.48 10E9/l
male	0.44 (0.006)	0.43 (0.004)	0.43 (0.006)	0.44 (0.006)	0.43 (0.007)	0.43 (0.005)	0.45 (0.006)	0.44 (0.007)	0.44 (0.005)	
female	0.41 (0.006)	0.42 (0.005)	0.41 (0.006)	0.40 (0.006) (n=18)	0.41 (0.005)	0.40 (0.004)	0.41 (0.006)	0.41 (0.004)	0.41 (0.005)	
Hemoglobin	8.91 (0.10)	8.96 (0.09)	8.92 (0.09)	8.91 (0.11) (n=38)	8.76 (0.09)	8.79 (0.08)	9.03 (0.10)	8.85 (0.10)	8.84 (0.10)	m: 8.2-11 10E9/l f: 7.3-9.7 10E9/l
male	9.23 (0.11)	9.23 (0.10)	9.21 (0.11)	9.32 (0.11)	9.01 (0.12)	9.12 (0.10)	9.36 (0.11)	9.21 (0.14)	9.21 (0.13)	
female	8.57 (0.13)	8.71 (0.12)	8.65 (0.11)	8.46 (0.12) (n=18)	8.54 (0.12)	8.48 (0.07)	8.67 (0.11)	8.52 (0.10)	8.49 (0.09)	

Table III. Vital signs and Total Side Effects. Means (and standard errors) of baseline, week 6 and week 12.

	Baseline			Week 6			Week 12		
	Placebo (n=39)	**300 mg PS** (n=40)	**600 mg PS** (n=41)	**Placebo** (n=39)	**300 mg PS** (n=40)	**600 mg PS** (n=41)	**Placebo** (n=39)	**300 mg PS** (n=40)	**600 mg PS** (n=40)
Diastolic Pressure	80.05 (1.82)	80.10 (2.40)	78.34 (2.15)	78.38 (1.67)	79.82 (2.37)	78.39 (2.16)	78.92 (1.65)	79.73 (1.76)	79.41 (1.72)
Male	80.15 (2.67)	78.89 (3.28)	79.90 (3.13)	78.65 (2.62)	80.42 (3.50)	78.50 (2.94)	78.40 (2.30)	79.79 (2.45)	80.20 (2.24)
Female	79.95 (2.52)	81.19 (3.55)	76.86 (3.01)	78.11 (2.11)	79.29 (3.30)	78.29 (3.23)	79.47 (2.44)	79.67 (2.57)	78.67 (2.65)
Systolic Pressure	130.82 (3.02)	133.50 (3.69)	128.56 (3.63)	127.44 (2.83)	133.00 (3.22)	131.39 (3.22)	125.41 (2.51)	128.93 (2.18)	131.07 (2.61)
Male	131.45 (4.36)	132.11 (5.50)	125.45 (4.70)	127.50 (4.32)	133.21 (5.38)	130.65 (3.81)	124.65 (3.63)	129.42 (3.26)	129.60 (3.27)
Female	130.16 (4.29)	134.76 (5.08)	131.52 (5.54)	127.37 (3.74)	132.81 (3.88)	132.10 (5.23)	126.21 (3.54)	128.48 (3.01)	132.48 (4.10)
Heart Rate	71.87 (1.76)	72.13 (1.74)	70.27 (1.58)	72.41 (1.87)	70.45 (1.43)	70.44 (1.22)	73.31 (1.54)	71.25 (1.49)	70.41 (1.92)
Male	71.90 (2.50)	71.00 (3.03)	66.80 (1.78)	69.15 (2.35)	70.58 (2.44)	66.85 (1.54)	72.25 (2.22)	69.00 (2.09)	65.75 (2.05)
Female	71.84 (2.53)	73.14 (1.91)	73.57 (2.41)	75.84 (2.78)	70.33 (1.66)	73.86 (1.58)	74.42 (2.15)	73.29 (2.05)	74.86 (2.92)
Total Side Effects	1.47 (0.05)	1.41 (0.05) (n=39)	1.42 (0.03) (n=40)	1.47 (0.05) (n=37)	1.41 (0.04) (n=39)	1.41 (0.04)	1.44 (0.05) (n=38)	1.37 (0.04) (n=38)	1.41 (0.04)
Male	1.43 (0.06)	1.39 (0.06) (n=19)	1.38 (0.05)	1.42 (0.05)	1.37 (0.06) (n=19)	1.40 (0.04)	1.41 (0.06)	1.30 (0.05)	1.41 (0.05)
Female	1.50 (0.07)	1.42 (0.07)	1.46 (0.05) (n=20)	1.53 (0.08) (n=17)	1.44 (0.06)	1.42 (0.06)	1.48 (0.08) (n=18)	1.43 (0.07) (n=19)	1.41 (0.06)

Since eleven biochemistry safety blood parameters were tested, α for ALAT after Bonferroni-Holme correction was set to 0.05/11=0.0045 for the first hypothesis. Therefore, after correction, the observation of increased ALAT is rendered non-significant.

Hematological parameters
No significant differences between treatment groups were found for the following hematological blood parameters: white blood count ($F_{2,111}$=2.97, ns), lymphocytes ($F_{2,111}$=0.02, ns), monocytes ($F_{2,111}$=0.47, ns), neutrophils ($F_{2,111}$=0.01, ns), platelet count ($F_{2,112}$=0.33, ns), basophiles ($F_{2,111}$=0.48, ns) and eosinophils ($F_{2,111}$=0.47, ns). Red blood count ($F_{2,110}$=3.26, p=0.042), hematocrit ($F_{2,112}$=3.07, p=0.050) and hemoglobin ($F_{2,112}$=3.82, p=0.025) decreased in both treatment groups compared to placebo after 12 weeks of treatment. However, neither of the latter three survived Bonferroni-Holme correction and hence were rendered insignificant. Ten hematology blood parameter hypotheses were tested, thus α was for hemoglobin 0.05/10=0.0050, for red blood count 0.05/9=0.0055 and for hematocrit 0.05/8=0.0063.

Vital Signs
There were no significant differences between treatment groups for diastolic pressure ($F_{2,113}$=0.50, ns) and heart rate ($F_{2,113}$=1.09, ns), whereas a significant difference of systolic pressure was found at week 12 ($F_{2,113}$=3.28, p=0.041). The systolic pressure of the placebo group decreased, and the PS 600 mg group increased after 12 weeks of treatment. However, this result was not significant, since three vital signs hypotheses were tested α for systolic pressure was 0.05/3=0.0167 after Bonferroni-Holme correction.

Subjective side effects
No significant difference was present for the summed total side effects (CHI^2(116)=1.392, df=2, ns).

Discussion

In summary, the results of this double-blind placebo-controlled study showed that S-PS did not influence biochemical and hematological safety parameters nor vital signs after a 12-weeks treatment of 300 or 600 mg. Subjective side effects also did not differ between the treatment groups.

The observed changes of ALAT (liver functioning), red blood count, hematocrit and hemoglobin, which disappeared after Bonferonni-Holme corrections were also not clinically relevant. Despite small fluctuations, values remained within normal clinical ranges. ALAT is an enzyme which catalyzes the reversible transamination reaction: L-alanine + α-ketoglutarate $\Leftrightarrow$ pyruvate + L-glutamate. This enzyme is mainly found in the liver and kidney and its activity increases with viral or toxic hepatitis. In a study on the tolerability of 300 mg oral BC-PS daily in 130 patients Cenacchi and colleagues (Cenacchi et al., 1987) showed that BC-PS induced significant reductions in uric acid and SGPT (also called ALAT), in male subjects. In our S-PS study no significant group differences were found for uric acid, while ALAT increased in the placebo group at week 12. Even if this difference would have been significant after the Bonferroni-Holme correction, our results indicate that PS treatment does not influ-

ence ALAT, because the ALAT concentration of the placebo group increased, while both treatment groups (300 and 600 mg S-PS) did not differ from the baseline values.

The clinical efficacy of this type of S-PS to improve cognitive function in subjects with age-associated memory is unclear until now. As mentioned before we recently reported that 300 and 600 mg S-PS did not influence cognitive functioning in subjects with age-associated memory impairment (Jorissen et al., 2001). In that article, we concluded that future studies should focus on the way of production of S-PS, because in our studies the S-PS content of the capsules degraded to 50% of the initial value after 15 months. According to Kidd (Kidd, 2000), daily supplementation with S-PS, minerals, the B vitamins (added in singly), omega-3 and omega-6 essential fatty acids, and flavonoids might also have cognitive efficacy in subjects with Attention Deficit/Hyperactivity Disorder.

However, we conclude that phosphatidylserine (S-PS) is a safe nutritional supplement for elderly if taken up to a dosage of 200 mg three times daily, but its efficacy remains to be demonstrated.

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6 Dietary Alpha-lactalbumin Increases the Ratio of Plasma Tryptophan to the other Large Neutral Amino Acids and Ameliorates Premenstrual Memory Consolidation Deficit

Mood and behavioral changes occurring 7-10 days before menses in women with Premenstrual Syndrome (PMS) may be related to abnormalities in serotonergic activity. To investigate, in a double-blind, placebo-controlled, cross-over study, the effects of a meal enriched with a high tryptophan whey protein concentrate rich in alpha-lactalbumin (α-lactalbumin), compared to a meal enriched with a low tryptophan protein (casein), on cognitive performance in women suffering from premenstrual symptoms. Fifteen women (18-45 years) experiencing premenstrual symptoms regularly for more than 2 years were tested on one postmenstrual and two premenstrual days. Performance on tasks of verbal memory, visual memory, and planning was measured. Blood samples were taken to assess the ratio of plasma concentration of tryptophan to that of the other large neutral amino acids. An increased ratio reflects increased tryptophan and serotonin availability in the brain. Visual memory was significantly worse in the premenstrual phase than in the postmenstrual phase. This visual memory impairment during the premenstrual phase was ameliorated by the α-lactalbumin meal but not by the casein meal. The plasma ratio of tryptophan to the other five large neutral amino acids increased significantly after the α-lactalbumin meal. The α-lactalbumin diet alleviated premenstrual visual memory deficits in women suffering from premenstrual symptoms, most likely by enhancing brain central serotonin synthesis.

Introduction

Premenstrual symptoms, such as mood swings, irritability, anxiety, breast tenderness, bloating, poor concentration and confusion, are common in women of reproductive age. Over 75% of women suffer from at least mild premenstrual symptoms (Ramcharan *et al.*, 1992), and some suffer from severe premenstrual symptoms, constituting Premenstrual Syndrome (PMS), or Premenstrual Dysphoric Disorder (PMDD), which is an extreme form of PMS that is diagnosed using several criteria (Mortola *et al.*, 1990; APA, 1994; Gold, 1994; Steiner, 2000). PMDD is much less common than PMS, with a prevalence of 5.8% and a cumulative lifetime prevalence of 7.4 % in young women aged 14 to 24 years (Wittchen *et al.*, 2002).
The mood and behavioral changes experienced by women with PMS have been attributed to abnormalities in central serotonergic activity (Freeman, 1996a). Moreover, changes in cognitive performance during the menstrual cycle may be related to changes in serotonergic functioning rather than directly to changes in hormone levels (Halbreich and Tworek, 1993), and there is evidence that PMS is related to abnormalities (i.e. reductions) in serotonergic functioning (Halbreich and Tworek, 1993). For instance, blood serotonin (5-HT) levels (Taylor *et al.*, 1984; Rapkin *et al.*, 1987) and platelet 5-HT uptake (Taylor *et al.*, 1984; Tam *et al.*, 1985; Ashby *et al.*, 1988) are lower in the premenstrual phase than in the postmenstrual phase in women with PMS. Pharmacological and neuro-endocrine challenge tests to assess 5-HT function in women with PMDD generally show impaired 5-HT function or lowered 5-HT responsiveness to, for instance, fenfluramine (FitzGerald *et al.*, 1997) and L-tryptophan (Bancroft *et al.*, 1991).
Studies employing cognitive assessments during the menstrual cycle has yielded controversial results (Sommer, 1992). Delayed recall of visual material is worse during the menstrual phase than during the luteal phase (Phillips and Sherwin, 1992), and the performance of tests of articulatory and fine motor skills is worse and that of tests of spatial ability is better during the menstrual phase than during the late follicular phase (Hampson, 1990; Hampson, 1995). However, several authors have not found menstrual cycle effects on the performance of tests of perception and recall of auditory presented sequences, visual spatial ability, verbal learning, verbal fluency and attention (Gordon and Lee, 1993; Nakatani *et al.*, 1993; Morgan and Rapkin, 2002; Owens *et al.*, 2002). Keenan and colleagues (Keenan *et al.*, 1995) also found no effect of menstrual cycle phase on several cognitive indices, including non-verbal memory and attention, but did find a phase-independent group difference between women with and without PMS on immediate (5^{th} presentation) and delayed verbal recall but not on recognition. In contrast to the study described in this paper, the above-mentioned studies were designed to correlate cognitive changes to ovarian steroid hormone levels during the menstrual cycle, and did not compare performance in the premenstrual and the postmenstrual phases in healthy volunteers or in women with confirmed PMDD, PMS or who at least reported premenstrual symptoms. In a study comparing the premenstrual and postmenstrual phase, time-pressure led to an impaired performance on Raven's Standard Progressive Matrices intelligence test during the premenstrual phase and improved it in the postmenstrual phase, but only in 64 female students with low trait anxiety and not in 64 students with high trait anxiety (Kumari and Corr, 1998). Morgan and colleagues (Morgan *et al.*, 1996) did not find any significant effects of group or cycle phase on attention and memory performance, or on a range of other cognitive functions in 30 women with PMS and 31 controls.

Drugs that promote 5-HT neurotransmission, such as selective serotonin reuptake inhibitors (SSRIs), are currently used in the treatment of PMDD (Steiner *et al.*, 1995; Dimmock *et al.*, 2000; Steiner, 2000). However, brain serotonin levels can also be manipulated by dietary interventions. Tryptophan, the precursor of serotonin, is an essential amino acid and can only be obtained from dietary protein (Crim, 1994). Dietary manipulations to influence central serotonin levels increase the availability of cerebral tryptophan by changing the ratio of tryptophan (TRP) to the sum of the 5 other large neutral amino acids (ΣLNAA), tyrosine, phenylalanine, leucine, isoleucine and valine. The plasma TRP/ΣLNAA ratio is increased after a carbohydrate-rich, protein-poor diet (Markus *et al.*, 1998) or carbohydrate-rich beverages (Sayegh *et al.*, 1995) and decreased after acute tryptophan depletion (ATD) (Biggio *et al.*, 1974; Riedel *et al.*, 1999; Schmitt *et al.*, 2000). This may reflect increased or decreased uptake of tryptophan through the blood-brain barrier and an increase or decrease in the central synthesis of serotonin, which responds rapidly to peripheral changes in tryptophan concentrations in animals (Fernstrom, 1977) and humans (Nishizawa *et al.*, 1997; Carpenter *et al.*, 1998). An alternative dietary manipulation to change the plasma TRP/ΣLNAA ratio and brain 5-HT is to include a whey protein concentrate rich in α-lactalbumin, which is a source high in tryptophan, in a standard diet (Markus *et al.*, 2000a; Markus *et al.*, 2000b; Markus *et al.*, 2002). The advantage of this manipulation is that the test (α-lactalbumin) and control (casein) conditions are isoenergetic – the diets contain equal amounts of carbohydrate, protein, and fat and do not differ in taste or appearance. Markus and colleagues (2000b) studied the influence of this α-lactalbumin diet on plasma TRP/ΣLNAA ratio, plasma prolactin concentration, and acute stress-induced changes in mood and cortisol in 29 high stress-vulnerable participants and 29 low stress-vulnerable controls. In another study the effect of the same manipulation on memory scanning performance was examined in 23 stress-vulnerable participants and 29 controls (Markus *et al.*, 2002). In both studies, the TRP/ΣLNAA ratio increased after the α-lactalbumin meal compared to the control casein meal. The α-lactalbumin diet prevented an acute stress-induced increase in depressive mood and cortisol level, increased plasma prolactin concentration (Markus *et al.*, 2000b), and increased memory scanning performance (Markus *et al.*, 2002) but only in stress-vulnerable participants (Markus *et al.*, 2000b). Thus a diet-induced increase in TRP/ΣLNAA may be beneficial in stress-prone individuals, whose brain 5-HT system is sensitized by continuous stress (Markus *et al.*, 1998; Markus *et al.*, 2000b; Markus *et al.*, 2002). Such stress-related vulnerability of the 5-HT system may also occur in women suffering from PMDD, who may also be more vulnerable in certain phases of the menstrual cycle (Bancroft *et al.*, 1991; Steiner, 1992).

Peripheral serotonin levels fluctuate during the menstrual cycle, with lower levels being measured in the premenstrual phase in women with PMS (Taylor *et al.*, 1984; Tam *et al.*, 1985; Rapkin *et al.*, 1987; Ashby *et al.*, 1988), which may explain the transient cognitive deficits of these women. The link between serotonin levels and cognitive functioning has been shown in individuals with Alzheimer's disease (Kumar *et al.*, 1995; Porter *et al.*, 2000) and in healthy volunteers (Riedel *et al.*, 1999; Schmitt *et al.*, 2000; Rubinsztein *et al.*, 2001). Thus inclusion of α-lactalbumin in the diet may be an alternative treatment for women with premenstrual symptoms, because such a diet increases 5HT levels. Moreover, such a diet may improve cognitive functioning via serotonergic effects in vulnerable participants. We suggest that in the premenstrual phase, women, and particularly those with premenstrual

symptoms, may be vulnerable to 5HT manipulations and therefore respond to such a nutritional intervention.
The present study was designed to investigate the effects of nutritional manipulation on 5-HT-related cognitive performance in women suffering from premenstrual symptoms. Two primary hypotheses and one secondary hypothesis were formulated. Firstly, we hypothesized, based on previous findings (Riedel *et al.*, 1999; Schmitt *et al.*, 2000; Rubinsztein *et al.*, 2001; Markus *et al.*, 2002), that administration of α-lactalbumin compared to the casein (as control treatment) would increase the TRP/ΣLNAA ratio and exert a specific positive effect on memory consolidation of both a pattern recognition task and a word recognition task during the premenstrual phase of the menstrual cycle. We also hypothesized that memory consolidation, immediate recall, and retrieval would be worse in the premenstrual phase than in the postmenstrual phase in women with at least moderate premenstrual symptoms. In order to investigate whether these putative memory effects of α-lactalbumin are associated with changes in executive function, our secondary hypothesis was that an α-lactalbumin diet would improve planning performance compared to the casein control diet. The mood and appetite outcome measures of this study are described in chapter 7.

Methods

Participants
Volunteers were recruited among university staff and students through advertisements in the University newspaper, and via posters on campus. Eighteen women suffering from premenstrual symptoms participated in the study. Inclusion criteria were self-reported premenstrual changes in mood, affect, well-being, and cognitive function, assessed by the Calendar Of Premenstrual Experience (COPE) (Mortola *et al.*, 1990); a reported history of these premenstrual complaints for more than 2 years; and a regular menstrual cycle. Exclusion criteria were oral contraceptive use; hormone replacement therapy; history of depressive disorder; pregnancy; breast-feeding; any medical disorder that could cause cognitive deterioration; excessive alcohol use; and current psychoactive medication. The participants were aged between 18 and 45 with a mean age (±SE) of 29 (±2). Participants were asked not to use any medication which they would normally use for their premenstrual symptoms for at least 2 days before the premenstrual test days or to take nutritional supplements during the study. The reproducibility of the occurrence of premenstrual symptoms was assessed for one complete menstrual cycle by means of Freeman's daily diary of premenstrual symptoms (Freeman *et al.*, 1996). The sum of the 17 symptoms increased in the premenstrual period by 48% compared to the postmenstrual period, and the increase was 44% for the mood subscale, 30% for the behavioral subscale, 69% for the pain subscale, and 76% for the physical symptoms subscale. The study was approved by the local ethics committee. All subjects gave a written informed consent prior to participation.

Study Design and Method
The study had a double-blind, placebo-controlled, cross-over design. The participants ate a meal and drank a chocolate drink containing either the whey protein concentrate rich in α-lactalbumin (treatment protein) or casein (placebo protein) on two premenstrual days (between day 22 and 28 of the menstrual cycle). Treatment order was counterbalanced. In addition, participants were tested in the postmenstrual phase of their menstrual cycle (between day 4 and 8), to assess postmenstrual baseline functioning. Premenstrual level of perform-

ance (defined as the average of the baseline assessments of the two premenstrual days) was compared to the postmenstrual level of performance. Eight participants underwent the two premenstrual test days in two separate cycles (pre-post-pre), while seven participants were tested on two premenstrual test days in the same cycle with at least 2 days in between (pre-post order), of which three in pre-pre-post order and four in post-pre-pre order. This means that there were 14 premenstrual test assessments before and 16 premenstrual test assessments after the 15 postmenstrual test assessments of the total of 45 test assessments. This indicates that the premenstrual-postmenstrual order was counterbalanced.

Table I. Composition and amino acid content of the chocolate drinks used in the α-lact[1] and casein meals.

	α-lact meal	Casein meal
Composition (g)		
α-Lactalbumin-enriched whey protein	20	0
Sodium caseinate	0	15.5
Cocoa	3.5	3.5
Granulated sugar	10	10
Water	200	200
Amino acid profile (g/kg)		
Isoleucine	27.61	31.80
Leucine	47.56	59.31
Phenylalanine	20.80	32.24
Tyrosine	16.82	33.13
Valine	29.52	44.09
Tryptophan	12.32	9.51
Trp:LNAA[2]	8.7	4.7

[1]α-lact meal, meal containing α-lactalbumin-enriched whey protein.
[2]Trp:LNAA, the ratio of tryptophan to the sum of the other large neutral amino acids.

Meals

The two meals were identical except for the composition of the chocolate drink: the treatment chocolate drink contained 20 g of whey protein concentrate rich in α-lactalbumin (Borculo Domo Ingredients, Borculo; The Netherlands), whereas the placebo chocolate drink contained 15.5 g of the tryptophan-poor protein sodium caseinate. Both chocolate drinks were identical to the drinks used in a previous study (Markus *et al.*, 2000b; Markus *et al.*, 2002) and were prepared by mixing chocolate powder, which was labeled with subject and test day number (1 or 2), with 200 ml water (see Table I for the amino acid profile of the chocolate drinks). Both meals consisted of 63.5% carbohydrate, 11.7% protein and 24.8% fat, with a total energy content of 6133 kilojoules (see Table II).

Biochemical assessments

Blood samples were collected into 4-ml lithium-heparin tubes and stored on ice. They were then centrifuged for 5 minutes at 4000 rpm at 4 ^{o}C within 30 minutes of collection. Subsequently, 100 μl plasma was deproteinized by vortexing it with 6 mg sulphosalicyclic acid (van Eijk *et al.*, 1994), and the deproteinized plasma was frozen in dry ice and stored at –70 ^{o}C until amino acid analysis by high-performance liquid chromatography (van Eijk *et al.*, 1993). The plasma TRP/ΣLNAA ratio was calculated by dividing plasma tryptophan (TRP) by the sum of the five other large neutral amino acids tyrosine, phenylalanine, leucine, isoleucine, and valine (ΣLNAA).

Table II. Components of the meal and its energy composition.

Diet	*Amount Used (g)*
Bread	70
Butter	30
Strawberry Jam	70
Red Grape Juice	500
Malt Bread	30
Mars bar	42
Chocolate Drink (α-lactalbumin or casein)	467
Percentage of energy total meal including α-lactalbumin or casein chocolate drinks (%)	
Protein	11.7
Fat	24.8
Carbohydrates	63.5

Cognitive assessments

The cognitive test battery consisted of tests of verbal and non-verbal learning, memory, and planning. The learning and memory tests have been selected due to their sentivity for serotonergic manipulations, which have been shown in previous ATD studies in healthy volunteers (Riedel *et al.*, 1999; Schmitt *et al.*, 2000; Rubinsztein *et al.*, 2001), whereas planning was not influenced by the serotonergic manipulation in healthy volunteers. All tests were administered by means of a computer, using E-prime for Windows. The computerized tests were presented on a 15-inch monitor and participants sat in an experimental cubicle approximately 50 cm from the monitor. The light in the experimental cubicle was turned on during the test assessments to maintain constant illumination. All participants were used to working with computers. In order to minimize learning effects, all participants underwent a training session before the test sessions. During this training session each volunteer completed all the cognitive tests.

Primary Cognitive Outcome measures

Abstract VIsual PAttern LEarning Task (Avipalet)

The Avipalet is a non-verbal pattern memory recognition task. A series of white on black patterns was presented three times on a computer screen. Each stimulus was presented for 3 seconds followed by an interval of 500 milliseconds. Participants were instructed to memorize the patterns as accurate as possible. Pattern recognition was tested immediately after the three trials and again after a 30-minute delay. The recognition trial consisted of 16 pairs of patterns which remained on the screen for 6 seconds or until the participant responded, followed by a 500-millisecond response-stimulus interval. Each pair consisted of a target and a distracter which were equally likely to appear on the left or right side of the screen. The median reaction time and the sensitivity (A', calculated in the same manner as for the VVLT) were dependent variables.

The Visual Verbal Learning Test (VVLT)

The VVLT measures short-term memory and long-term memory performance and is adapted from the Rey Auditory Verbal Learning Test (Rey, 1964). The test consisted of a list of 30 monosyllabic words matched for frequency, imagery, and concreteness. The word list was presented in the same sequence in three trials on a computer screen at a rate of one word every 2 seconds. Each presentation ended with a free verbal recall of the words (immediate

recall). The sum of the recall of the three trials was calculated. Thirty minutes after the third presentation, the participant was requested to recall as many words as possible from the list she had seen earlier (delayed recall). A recognition test, which consisted of 15 formerly learned words (targets) and 15 new (distracters), was given after the delayed recall test. After presentation of each word the participant had to respond as fast as possible by pressing either a “YES” or “NO” button on the keyboard to indicate whether she recognized the word from the previous list. The words remained on the screen for 2 seconds or until the participant responded; 1 second elapsed before the next word appeared on the screen. According to the theory of signal detection (Pollack and Norman, 1964), the proportion of correctly recognized words (cr) and the proportion of falsely recognized (fr) constitute the non-parametric sensitivity measure: A’ = 1 - 1/4 (fr/cr + (1-cr)/(1-fr)). A’ is in fact the proportion of correctly recognized words, corrected for the participant's response tendency. Because the distribution of A’ is skewed due to a ceiling effect, A’ was arc sin transformed before statistical analysis.

The outcome variables were the total number of correct words recalled during the three immediate recall trials as a measure of short-term memory; the number of correct words produced on delayed free recall as a measure of retrieval from long-term memory; A’ as a measure of storage in long-term memory; and the median reaction time (RT) of correctly recognized target words as a measure of speed of retrieval from long-term memory. Delayed recall and delayed recognition of the list learned at baseline were tested a second time during the test assessment that started 4 hours after start of the treatment, which is 4 hours and 50 minutes after the presentation of the words. In each of the assessments, parallel versions of the word lists were presented. The order of the lists was balanced across the participants.

Secondary cognitive outcome measures

Tower of London (TOL)

Planning capacity was assessed by using a modified version of the One-touch Tower of London (TOL) task (Owen *et al.*, 1995). During the training session, participants were first told the rules of the original non-computerized TOL (Shallice, 1982). Thereafter, the computerized TOL was introduced. On a computer screen, two arrays of differently coloured balls (red, yellow and blue) on sticks were presented. The participant was requested to indicate the minimal number of steps (2-6) necessary to rearrange three coloured balls on the top configuration to match the arrangement presented on the lower half of the screen, by pressing the appropriate response button. The tests consisted of 40 trials, with an equal number of 2-, 3-, 4- and 5-step problems, which were presented in a fixed pseudo-random order. To avoid participants responding automatically with a 5-step response when the problem seems to be too complex, four 6-step trials were added, the results of which were not used for data analysis. Parallel versions of the test were used for each assessment. The order of the versions was rotated over the participants. A new analytical procedure was applied to reduce the set of Response Time data across levels of difficulty. Transformation and regression functions of RT on the number of steps were determined to obtain slope and intercept coefficients, based on previous data obtained from groups of 20 young- and 20 elderly volunteers (Riedel, 2000). Briefly, each RT value was transformed according to the equation: y = sqrt(10log(x)), so that transformed RTs are a linear function of the number of steps. Regression analyses were then carried out on transformed RTs to yield individual slopes and intercepts of the RT on steps function. Slope is considered the primary outcome variable measuring problem solving ability. Intercept is a variable measuring response speed unrelated to

problem solving ability. As no consistent association has been established between number of errors and the number of steps, the total number of errors is the other outcome variable.

Procedure

The participants were asked not to drink alcohol on the day before the experiment, and not to eat or drink (except water) after 10 pm that evening, and to arrive at the laboratory well rested. Ovulation detection kits were used to determine an appropriate date for the premenstrual assessments (about 10 days postovulation, adjusted for usual length of the cycle). After arrival on the test days, the cognitive test battery was completed (t -1) and then blood samples were taken at baseline (t0). The participants then ate one of the two meals. The total diet (see Table II) was served as breakfast, snack and lunch. Breakfast and lunch consisted of brown bread with butter and jam, malt loaf (Soreen) with butter, red grape juice, and the chocolate drink, and the snack was a chocolate bar (Mars) served with a glass of red grape juice. The cognitive test assessment was repeated 4 hours (t+4) after the start of the treatment. Blood samples were collected 3.5 (t+3½) and 5 (t+5) hours after the start of treatment. The participants were also asked to fill in a daily diary during their participation in the study (Freeman *et al.*, 1996b). Participants were allowed to watch videos, read books or work in their office on campus.

Statistical Analysis

All statistical analyses were performed with SPSS 10.0 for Windows. To assess treatment-related differences, the cognitive outcome variables on each test day were analysed using a General Linear Model (GLM) for repeated measures. Within-subjects factors were treatment (two levels: α-lactalbumin diet, casein diet) and time (two levels: t-1, t+4). We hypothesized that treatment would affect cognitive performance in the premenstrual phase. Hence the time x treatment interaction was the only effect of interest. Basically, this is an effect of the nutritional manipulation and is referred to as the effect of nutritional manipulation in the subsequent text. The ‘menstrual cycle’ effect was analysed as the within-subject effect of the two premenstrual baseline assessments (t-1) and the postmenstrual assessment (two levels: premenstrual, postmenstrual). A 2 by 3 GLM was used to analyse the biochemical outcome measures with two levels of treatment (α-lactalbumin diet, casein diet) and three levels of time (t0, t3½, and t5) as within subject factors.

To examine whether cycle phase order or treatment order influenced the results, these factors were added as between subject factors in separate analyses. Since no effects of order were found, the results of the GLM without these between subject factors are presented.

The primary statistical analysis followed the recommendation of Kepple (Kepple, 1991) and was carried out using planned comparisons, which were based on previous findings for the same and other serotonergic manipulations and cognitive performance (Riedel *et al.*, 1999; Rubinsztein *et al.*, 2001; Markus *et al.*, 2002).

Results

Fifteen women completed the study. One participant withdrew after completing only the casein treatment because she suffered from pneumonia during her next premenstrual phase. Two volunteers did not continue after test training due to irregularity of their menstrual cycle. The TOL data from one participant was not used in the analysis because she failed to complete the task. Of the fifteen participants, only twelve agreed to provide blood samples.

Cognitive assessments

The results of the outcome variables are listed in Table III. The Avipalet immediate and delayed recognition reaction time results are shown in Figure I.

Primary outcome variables

Avipalet

The Avipalet delayed recognition reaction time ($F_{1,14}$=6.02, p<0.05) improved significantly after the α-lactalbumin meal compared to the casein meal. However, no effects of nutritional manipulation were found for Avipalet immediate recognition sensitivity, Avipalet delayed recognition sensitivity, or Avipalet immediate recognition reaction time.

Furthermore, Avipalet immediate recognition sensitivity and delayed recognition sensitivity did not differ between the postmenstrual and premenstrual test sessions, whereas Avipalet immediate recognition reaction time ($F_{1,14}$=5.86, p<0.05) and the Avipalet delayed recognition reaction time ($F_{1,14}$=8.37, p<0.05) were significantly faster in the postmenstrual session than in the premenstrual session.

Visual Verbal Learning Test

Nutritional manipulation did not affect VVLT immediate recall, delayed recall, second delayed recall, recognition, or second delayed recognition performance. Immediate recall showed a main effect of menstrual cycle ($F_{1,14}$=7.66, p<0.05), with performance being better in the postmenstrual phase than in the premenstrual phase. Delayed recognition reaction time was faster in the postmenstrual phase than in the premenstrual phase (F=1,14=16,05, p<0.005). Phase of menstrual cycle did not affect VVLT delayed recall, second delayed recall, delayed recognition, or second delayed recognition performance.

Figure I. Mean (+ se) ratios of plasma tryptophan compared to the sum of the other large neutral amino acids (TRP/ΣLNAA) at baseline (t_0), 3.5 ($t_{3½}$) and 5 (t_5) hours after the start of the treatment. Lines reflect values obtained during the premenstrual phases after the TRP-enriched meal alfalact and placebo meal (casein). The single reference value obtained during the postmenstrual phase is depicted as a triangle at t_0.

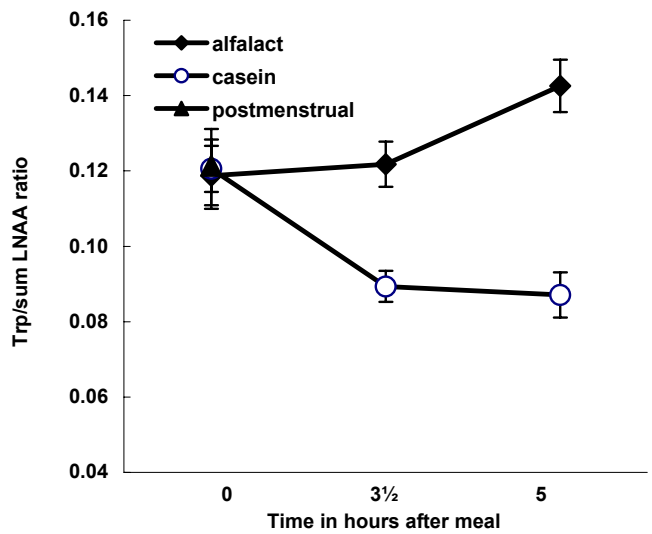

Table III. Means (Standard errors) of the outcome variables at baseline (t -1) and 4 hours after the serving the meals (t4) at the premenstrual (pre) assessments. The postmenstrual (post) cognitive assessment was only at baseline (t -1). The assessment 't -1 → t4' denotes the second delayed recall recognition of the VVLT word list learned at baseline (* p<0.05, ** p<0.01, *** p<0.005, **** p<0.001).

Visual Verbal Learning Test	*Treatment*	*Pre t -1*	*Pre t -1→ t4*	*Pre t4*	*Post*	*Time*	*Treat x Time*	*Cycle (t-1pre versus post)*
VVLT Delayed Recall (words)	Alfalact	18.0 (1.3)	16.1 (1.1)	15.2 (1.5)	18.4 (1.5)	$F_{1,14}=12.67^{***}$	$F_{1,14}=0.25$	$F_{1,14}=0.005$
	Casein	18.7 (1.3)	16.9 (1.5)	14.9 (1.6)		F1,14=0.86 (recall t -1→ t4)		
VVLT Delayed Recognition (sensitivity)	Alfalact	96.3 (1.0)	91.6 (1.9)	95.2 (0.8)	96.9 (0.8)	$F_{1,14}=5.23^{*}$	$F_{1,14}=0.04$	$F_{1,14}=0.004$
	Casein	97.3 (0.8)	92.3 (1.6)	96.2 (0.8)		$F_{1,14}=0.17$ (recog t -1→ t4)		
VVLT Delayed Recognition (reaction time)	Alfalact	682 (24)	745 (30)	719 (25)	633 (18)	$F_{1,14}=1.15$	$F_{1,14}=3.41$	$F_{1,14}=16,05^{***}$
	Casein	692 (16)	721 (29)	686 (21)		$F_{1,14}=0.59$ (recog t -1→ t4)		
VVLT Immediate Recall (words)	Alfalact	50.6 (3.6)		51.5 (2.5)	57.2 (2.65)	$F_{1,14}=0.95$	$F_{1,14}=3.70$	$F_{1,14}=7.66^{*}$
	Casein	53.2(2.6)		48.7 (3.4)				
Avipalet	*Treatment*	*Pre t -1*	*Pre t -1→ t4*	*Pre t4*	*Post*	*Time*	*Treat * Time*	*Cycle (t-1pre versus post)*
Avipalet Delayed Recognition (sensitivity)	Alfalact	88.9 (3.0)		91.9 (1.7)	92.2 (2.0)	$F_{1,14}=0.33$	$F_{1,14}=0.18$	$F_{1,14}=1.03$
	Casein	91.0 (3.4)		90.9 (2.6)				
Avipalet Delayed Recognition (reaction time)	Alfalact	1722 (125)		1538 (69)	1447 (98)	$F_{1,14}=0.10$	$F_{1,14}=6.02^{*}$	$F_{1,14}=8.37^{*}$
	Casein	1555 (86)		1706 (111)				
Avipalet Immediate Recognition (sensitivity)	Alfalact	93.7 (1.9)		90.4 (2.2)	94.8 (1.3)	$F_{1,14}=0.56$	$F_{1,14}=0.94$	$F_{1,14}=0.24$
	Casein	93.0 (2.1)		94.7 (0.9)				
Avipalet Immediate Recognition (reaction time)	Alfalact	1630 (102)		1545 (74)	1412 (76)	$F_{1,14}=0.21$	$F_{1,14}=0.39$	$F_{1,14}=5.86^{*}$
	Casein	1514 (98)		1528 (79)				
Tower of London	*Treatment*	*Pre t -1*		*Pre t4*	*Post*		*Treat * Time*	*Cycle (t-1pre versus post)*
CompTOL (slope)	Alfalact	0.14 (0.01)		0.15 (0.01)	0.15 (0.01)	$F_{1,13}=0.19$	$F_{1,13}=0.17$	$F_{1,13}=0.32$
	Casein	0.15 (0.01)		0.15 (0.01)				
CompTOL (intercept)	Alfalact	3.50 (0.03)		3.47 (0.02)	3.46 (0.03)	$F_{1,13}=1.72$	$F_{1,13}=0.20$	$F_{1,13}=1.77$
	Casein	3.46 (0.04)		3.44 (0.04)				

Secondary outcome variables

Comp-TOL

No significant meal or cycle differences were found for the Comp-TOL slope and the Comp-TOL intercept.

Biochemical variables

Nutritional manipulation ($F_{2,10}$=8.84, $p < 0.01$) significantly affected the plasma TRP/ΣLNAA ratio. The plasma ratio was 38% higher at t3½ ($F_{1,11}$=6.181, $p<0.05$) and 70% higher at t5 ($F_{1,11}$=17.471, $p<0.005$) (see Figure I and Table IV) after the α-lactalbumin meal than after the casein meal. The plasma TRP/ΣLNAA ratio increased by 6% at t3½ and 25% at t5 compared to baseline after the α-lactalbumin diet, but decreased by 22% at t3½ and by 25% at t5 compared to baseline after the casein diet. There was no main effect of cycle for the plasma TRP/ΣLNAA ratio.

Table IV. Tryptophan (TRP) concentration and the other long chain neutral amino acids in µmol/l, the concentration of the sum of the large neutral amino acids (∑LNAA) in µmol/l and the TRP/∑LNAA ratio at t_0, $t_{3½}$ and t_5 for the premenstrual test sessions and the postmenstrual test session.

		t_0	$t_{3½}$	t_5
Premenstrual Treatment (Alfalact meal)	Tryptophan	50.07 (3.3)	71.71 (4.1)	71.47 (4.6)
	Iso-Leucine	55.74 (2.4)	105.37 (5.2)	85.37 (4.6)
	Leucine	91.03 (2.5)	142.70 (7.8)	108.83 (6.0)
	Phenyl-Alanine	54.05 (1.8)	68.26 (2.3)	67.17 (2.7)
	Tyrosine	43.22 (1.4)	57.09 (3.5)	49.70 (1.6)
	Valine	179.87 (6.2)	218.54 (10.2)	191.81 (8.3)
	∑LNAA	423.90 (10.8)	591.96 (27.1)	502.89 (21.1)
	TRP/∑LNAA ratio	0.12 (0.0)	0.12 (0.0)	0.14 (0.0)
Premenstrual Placebo (Casein meal)	Tryptophan	49.40 (4.1)	47.43 (0.1)	63.47 (4.3)
	Iso-Leucine	54.40 (2.3)	75.82 (7.0)	109.19 (8.7)
	Leucine	89.90 (3.4)	114.30 (12.2)	169.31 (13.9)
	Phenyl-Alanine	53.17 (2.1)	69.31 (4.5)	85.87 (4.0)
	Tyrosine	42.65 (1.1)	66.41(7.9)	97.05 (9.0)
	Valine	172.09 (6.5)	211.52 (17.2	284.83 (18.0)
	∑LNAA	412.22 (11.8)	537.36 (47.9)	746.25 (51.1)
	TRP/∑LNAA ratio	0.12 (0.0)	0.09 (0.0)	0.09 (0.0)
Postmenstrual	Tryptophan	53.75 (4.1)		
	Iso-Leucine	60.60 (5.6)		
	Leucine	97.45 (7.4)		
	Phenyl-Alanine	57.05 (3.4)		
	Tyrosine	49.55 (2.8)		
	Valine	179.64 (10.8)		
	∑LNAA	444.31 (26.8)		
	TRP/∑LNAA ratio	0.12 (0.0)		

Discussion

In women suffering from premenstrual symptoms, Avipalet long-term visual pattern recognition reaction time in the premenstrual phase was significantly faster after an α-lactalbumin meal than after a casein meal. Moreover, the plasma TRP/ΣLNAA ratio increased significantly after the α-lactalbumin meal than after the control casein meal. Further, menstrual cycle phase influenced VVLT immediate recall performance, VVLT delayed recognition reac-

tion time, Avipalet immediate recognition reaction time, and Avipalet delayed recognition reaction time. For all these variables, performance was better during the baseline postmenstrual session than in the baseline premenstrual test sessions.

Markus and colleagues (Markus *et al.*, 2000b; Markus *et al.*, 2002) reported an increase in the TRP/ΣLNAA ratio of 43% or 48% at t=4 after an α-lactalbumin meal compared to a casein meal, whereas we found that the ratio was increased by 38% at t=3½ and 70% at t=5 (See Figure I). The greater ratio at t=5 in this study suggests that the maximum increase in TRP/ΣLNAA ratio after an α-lactalbumin meal had not yet been reached, and it would be interesting to establish the maximum and optimum increase in future studies. Our results showed that not only did the ratio increase after the α-lactalbumin meal, but also that the ratio decreased after the casein diet relative to baseline values. This prompts the question whether the casein meal is an appropriate placebo. The decrease in the TRP/ΣLNAA ratio after the casein meal in our study was comparable to that seen after ingestion of a balanced meal (Spring *et al.*, 1989). This suggests that the casein meal was an appropriate placebo. The α-lactalbumin-induced change in the TRP/ΣLNAA ratio was accompanied by an effect on memory, whereas there is no relation between the TRP/ΣLNAA ratio and premenstrual versus postmenstrual memory differences. This suggests that the TRP/ΣLNAA ratio is a good marker for the cognitive effects of nutritional manipulation. Women suffering from premenstrual symptoms appeared to be vulnerable to serotonergic changes during the premenstrual phase. This is in line with the results of Markus and colleagues (Markus *et al.*, 2002), who found an effect of α-lactalbumin only in high stress-prone subjects, who are also vulnerable to serotonergic changes.
Interestingly, the increase in the TRP/ΣLNAA ratio after consumption of the α-lactalbumin meal is as impressive as the 79% decrease in the plasma TRP/ΣLNAA ratio 5 hours after ATD (Schmitt *et al.*, 2000).The α-lactalbumin manipulation differs from treatment with exogenous tryptophan (Steinberg *et al.*, 1999) in that α-lactalbumin was part of a meal with a total protein content not higher than 11%, whereas Steinberg et al. did not control the protein content of the total meal. This may have lead to a fluctuating TRP/ΣLNAA ratio during the exogenous tryptophan treatment. Thus an α-lactalbumin manipulation may lead to a higher tryptophan concentration compared to that of the other large neutral amino acids.

Post-hoc analyses showed a significant Pearson's Correlation (R=-0.64; p=0.024; n=12) between the treatment-placebo difference in delayed pattern recognition reaction time after the treatment at t_5 and the difference of the TRP/ΣLNAA ratio at the same time point. There were no significant correlations between the other primary cognitive outcome variables and the TRP/ΣLNAA ratio treatment differences at t_5. Further there were no meaningfull or significant correlations between the t_5-t_0 (day) difference in TRP/ΣLNAA ratio's and the t_5-t_0 differences of the primary cognitive outcome variables. These latter correlations may not be a suitable indicator for this treatment, since there is not only an increase in TRP/ΣLNAA ratio after the alfalact meal, but also a decrease after the casein meal.

Changes in cognitive performance during the menstrual cycle have been studied before but results are contradictory. Maki and colleagues (Maki *et al.*, 2002) and Postma and colleagues (Postma *et al.*, 1999), reported for instance contradicting results. These studies did not compare the premenstrual (day 22 – 28 of the cycle) and the postmenstrual (day 4 – day 8) phases, but studied the cognitive differences between the phases with different hormone

levels. The first study compared the midluteal phase with the early follicular phase (or menses), whereas the latter compared the ovulation phase with the early follicular phase. The choice of cycle phase to test cognitive performance is crucial and may explain the contradictory results obtained. Thus it is important to compare results of studies with the cognitive tests assessments scheduled in the same cycle phases. Our study compared the premenstrual with the postmenstrual phase of the menstrual cycle. Man and colleagues (Man *et al.*, 1999) studied premenstrual versus postmenstrual performance differences in 10 women with PMDD and 10 control women and found a group-independent cycle effect for spatial working memory. The performance of both groups decreased during the late luteal phase (premenstrual) compared to the follicular phase (postmenstrual), which is comparable with the results of the study presented here. Interestingly, spatial working memory was influenced by phase of the cycle in both groups of women (Man *et al.*, 1999), whereas clinical depression during the luteal phase was only present in the PMDD group, indicating that mood was not influenced by cycle in the control group. This is in line with the results of ATD studies in healthy volunteers (Riedel *et al.*, 1999; Schmitt *et al.*, 2000; Rubinsztein *et al.*, 2001). ATD only influenced memory functions and not measures of mood. The above-mentioned studies and our study compared the effects of hormonal changes or the effects of serotonergic changes related to the menstrual cycle on cognitive performance. Since it has been shown that serotoninergic neurons in non-human primates contain oestrogen β and progestin receptors, and thus integrate steroid hormone effects in their action in the central nervous system (Bethea *et al.*, 2002), cognitive changes throughout the menstrual cycle should not be solely related to hormonal or serotonergic changes, but rather to a combination of both.

Regarding the cognitive effects of nutritional manipulation, Rubinsztein and colleagues (Rubinsztein *et al.*, 2001) showed a selective impairment of performance of a comparable pattern recognition task after the plasma TRP/ΣLNAA ratio was decreased by ATD in healthy volunteers, whereas other authors found a selective impairment of VVLT memory consolidation (Riedel *et al.*, 1999; Schmitt *et al.*, 2000). These latter memory consolidation effects were ascribed to the central serotonergic changes. We found a selective improvement of Avipalet pattern recognition memory reaction time, but not of the VVLT delayed recognition reaction time, after the TRP/ΣLNAA ratio was increased in women vulnerable to serotonergic changes. However, both variables were influenced by the phase of the menstrual cycle. Task accuracy, reflected by “sensitivity” of Avipalet and VVLT, was not influenced by treatment. The α-lactalbumin meal improved only the reaction time of the more complex spatial task. The pattern recognition task is probably more sensitive than the VVLT to acute serotonergic manipulation, given that cycle phase differences in easier tests of executive functions are not likely to be mediated by acute serotonergic changes. Our results show that the α-lactalbumin meal did not influence planning performance measured with the Tower of London, and as such are in line with those of another study (Schmitt *et al.*, 2000), in which ATD did not affect planning performance in this test in healthy volunteers. This study did not show a cycle phase difference in planning performance, and thus an improvement after α-lactalbumin treatment, considering that the women performed in the premenstrual and the postmenstrual phase on the same level, was not expected.

The possibility that cognitive premenstrual symptoms may be related to serotonergic changes is a popular idea. Sayegh and colleagues (Sayegh *et al.*, 1995) studied the efficacy of a carbohydrate-rich beverage on mood, appetite, and cognitive function. The drink de-

creased self-reported depression, anger, confusion, and carbohydrate craving, whereas word recognition performance improved compared to performance during the placebo run-in month. The carbohydrate drink increased the plasma TRP/ΣLNAA ratio and probably increased central serotonin levels (Sayegh *et al.*, 1995), based on the assumption that central tryptophan availability increases central serotonin availability, as has been observed in rat studies (Fernstrom, 1977). Our results point in the same direction, since the α-lactalbumin meal increased the TRP/ΣLNAA ratio in women suffering from premenstrual symptoms. These women appear to be vulnerable to serotonergic changes, since their whole blood serotonin (5-HT) levels (Taylor *et al.*, 1984; Rapkin *et al.*, 1987) and platelet 5-HT uptake (Taylor *et al.*, 1984; Tam *et al.*, 1985; Ashby *et al.*, 1988) are lower in the premenstrual phase than in the postmenstrual phase. Markus and colleagues (Markus *et al.*, 2000b) showed an increase in the plasma tryptophan level and a higher prolactin response after the α-lactalbumin diet than after the casein diet, but only in highly stress-vulnerable participants. The prolactin response makes it very likely that the α-lactalbumin diet indeed induced an increase in central serotonin levels.

A limitation of our study is that the occurrence of the premenstrual symptoms was assessed prospectively for only one menstrual cycle, using Freeman's daily diary of premenstrual symptoms (Freeman *et al.*, 1996b). To confirm PMS it is necessary to monitor two menstrual cycles, using this instrument. However, in this study premenstrual symptoms increased by more than 30% (30-76% for the different subscales) 6 days before menses compared to the symptom scores in the intermenstrual period (days 5-10 of the cycle). This level of symptoms is consistent with that reported for women suffering from PMS (Gold, 1994). Another weakness of this study is that the effects of α-lactalbumin were only studied in the premenstrual phase and not in the postmenstrual phase. Thus we do not know whether α-lactalbumin meal alleviates cognitive problems in other phases of the menstrual cycle. The treatment may also improve cognitive performance in the postmenstrual phase. Further, the blood samples were not used to analyse the oestrogen and progesterone levels, which could have been helpful in determining an appropriate date for the premenstrual assessments. For this purpose ovulation detection kits were used instead. Ovulation detection kits are not as precise as hormone blood samples, but during the postmenstrual test sessions the premenstrual symptoms diaries were checked to find out whether the premenstrual test session was indeed planned not more than 7 days before menses (day 22-28). The sample size of this study could also be questioned. However, a power analysis of a previous study with a similar dietary manipulation showed an effect size of over 0.8. For an effect which explains 30% of the variance with two conditions (df = 1), a sample size of 21 was required. The effect anticipated was more than double therefore the sample size of 15 with a within-subjects design should be adequate.

This study demonstrated that α-lactalbumin dietary manipulation may be a beneficial nutritional treatment for premenstrual memory deficits in women suffering from premenstrual symptoms, although these results should be replicated in other studies. Since conventional treatments for more severe premenstrual symptoms are accompanied by side effects, future studies should compare the dietary manipulation with treatment with SSRIs in women with prospectively confirmed PMDD or PMS and in women without symptoms.

In summary, the delayed recognition visual pattern reaction time improved specifically after ingestion of an α-lactalbumin meal compared to a casein meal. Further, immediate and delayed recognition reaction time performance in a visual pattern memory task and total immediate recall performance in a word learning task were better in the postmenstrual than in the premenstrual phase in women suffering from premenstrual symptoms.

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7 The Effect of Alpha-lactalbumin on Mood and Appetite in Women Suffering from Premenstrual Symptoms

Premenstrual symptoms such as low mood and increased appetite have been linked to abnormalities in serotonergic activity. We report the results of a double-blind, placebo-controlled, cross-over study of the effects of a meal enriched with a high tryptophan whey protein concentrate rich in alpha-lactalbumin (α-lactalbumin), compared to a meal with casein on the ratio of plasma tryptophan to the sum of the other large neutral amino acids (TRP/ΣLNAA), mood and appetite, in women suffering from premenstrual symptoms. Fifteen women (18-45 years), reporting premenstrual symptoms for more than 2 years, were tested on one postmenstrual and two premenstrual days. Visual Analogue Scales (VAS) of positive and negative mood and appetite, the Positive And Negative Affect Scales (PANAS), and the TRP/ΣLNAA ratio, reflecting increased brain serotonin availability, were measured. Participants also completed a food diary for six days to assess total daily energy intake and the nutrient content of their diet. There was a significant decline in mood and affective state during the premenstrual baseline test sessions compared with the postmenstrual test session. Participants were more anxious and irritable during, and less alert after the premenstrual baseline test sessions compared to the postmenstrual test day. The α-lactalbumin meal significantly improved mood and increased the TRP/ΣLNAA ratio during the premenstrual phase compared to the casein meal. The α-lactalbumin meal had no effects on appetite and food intake in the premenstrual phase of the cycle, and there was no fluctuation in food intake and appetite throughout the menstrual cycle.

Introduction

Over 75% of women suffer from premenstrual symptoms, such as mood swings, irritability, anxiety, breast tenderness, bloating, of at least a mild degree (Ramcharan *et al.*, 1992). Some of these women suffer from severe premenstrual symptoms, constituting Premenstrual Syndrome (PMS), or Premenstrual Dysphoric Disorder (PMDD), which is an extreme form of PMS, diagnosed using several different diagnostic criteria (Mortola *et al.*, 1990; APA, 1994; Gold, 1994; Steiner, 2000). PMDD is less common than PMS in women and has a baseline prevalence of 5.8% and a cumulative lifetime prevalence of 7.4% according to a recent prospective longitudinal community survey study using DSM IV criteria (Wittchen *et al.*, 2002).

The mood and behavioral changes, which occur 7-10 days before menses in women with PMS and PMDD, may be mediated by abnormalities in central serotonin (5-Hydroxy-Tryptamine; 5-HT) activity (Halbreich and Tworek, 1993; Freeman, 1996). Hence, changes in affective state (mood swings, depression, irritability), physical alterations (breast tenderness, bloating, weight gain) and appetitive symptoms (increased food intake, food cravings) seen in PMS are consistent with the involvement of serotonin (Freeman, 1996). In addition, lower whole blood 5-HT levels (Rapkin *et al.*, 1987) and reduced platelet 5-HT uptake has been demonstrated in PMDD (Ashby *et al.*, 1988) and during the late luteal phase, pharmacological and neuro-endocrine challenge tests to assess 5-HT function in these participants generally show impaired 5-HT function or lowered 5-HT responsiveness to for instance fenfluramine (FitzGerald *et al.*, 1997) and L-tryptophan challenge (Bancroft *et al.*, 1991). Also, pharmacotherapies aimed to increase 5-HT function by means of continuous or intermittent treatment with selective serotonin reuptake inhibitors (SSRI's) (Steiner *et al.*, 1995; Dimmock *et al.*, 2000), or through subchronic tryptophan loading (Steinberg *et al.*, 1999) has been shown to alleviate at least some of the affective symptoms of PMDD.

Changes in appetite during the menstrual cycle have also been attributed to abnormalities in 5-HT activity (Wurtman, 1990; Freeman, 1996). Enhanced carbohydrate intake induces an increase of glucose and insulin, followed by the uptake of the large neutral amino acids (LNAA's) tyrosine, phenyl-alanine, leucine, iso-leucine and valine into the skeletal muscles, whereas insulin prevents tryptophan uptake into the muscles. This results in an increase of the TRP/ΣLNAA plasma ratio, which gives tryptophan, the only precursor of 5-HT, an advantage in the competition with the other LNAA's to pass the blood brain barrier (Fernstrom and Wurtman, 1971b; Fernstrom and Wurtman, 1971a; Fernstrom, 1977). Since central serotonin synthesis responds rapidly to changes in precursor availability in animal brain (Fernstrom, 1977) and human brain (Nishizawa *et al.*, 1997; Carpenter *et al.*, 1998), the result is an increase in 5-HT synthesis in the brain. It has been suggested that carbohydrate craving and consumption during the premenstrual phase (Brzezinski *et al.*, 1990), which stimulates brain 5-HT synthesis, may be a form of self medication in order to improve mood (Wurtman *et al.*, 1989). Experimental data suggest that consumption of carbohydrates can indeed decrease depressive feelings, anger and confusion and improve memory functions in women with PMS (Sayegh *et al.*, 1995). Treatments and interventions which lead to an increase in central 5-HT concentration reduce food consumption, while interventions which decrease 5-HT activity bring about the opposite effect (Blundell, 1977). Food intake and food cravings fluctuate during the menstrual cycle and changes in appetite may be greatest in women who are vulnerable to premenstrual mood changes (Dye and Blundell, 1997).

In this study we use a carefully controlled meal containing whey protein concentrate rich in alpha-lactalbumin (α-lactalbumin). α-lactalbumin is a whey protein with the highest tryptophan content of all food protein sources (Heine et al., 1996). It has previously been demonstrated that this protein causes a significant increase in plasma TRP/ΣLNAA ratio and improves mood and performance in vulnerable subjects (Markus *et al.*, 2000a; Markus *et al.*, 2000b; Markus *et al.*, 2002). In addition, α-lactalbumin supplementation may be a good nutritional alternative for women with less severe premenstrual symptoms than PMDD, and may be more acceptable than pharmacological (e.g. SSRI) treatments.

In this study we investigate the effects of α-lactalbumin versus casein (a protein that has a low TRP content), in the premenstrual phase of the menstrual cycle in women with premenstrual symptoms, on mood and appetite. Specifically, we hypothesize that mood is impaired, and appetite and energy intake is increased in the premenstrual phase as compared to the postmenstrual phase of the menstrual cycle in women with at least moderate premenstrual symptoms. In addition we examine whether CHO consumption specifically is increased. Furthermore, we hypothesize that mood will improve and appetite will decrease after an α-lactalbumin treatment compared to the casein control treatment, during the premenstrual phase of the menstrual cycle in the same women. The cognitive outcome measures of this study are described in chapter 6.

Methods

Participants
Volunteers were recruited from university staff and students through advertisements in the University newspaper, and through posters on campus at the University of Leeds. Eighteen women suffering from premenstrual symptoms participated in the study. Inclusion criteria were self-reported premenstrual subjective changes in mood, affect, well-being and cognitive function, assessed by means of the Calendar Of Premenstrual Experience (COPE) (Mortola *et al.*, 1990), a reported history of these premenstrual complaints for more than 2 years and a regular menstrual cycle. Exclusion criteria were oral contraceptive use, Hormone Replacement Therapy, history of depressive disorder, pregnancy, breast feeding, any medical disorder that could produce cognitive deterioration, excessive alcohol use and current medication. The participants were aged between 18 and 45 years with a mean age (±SE) of 29 (±2). Participants were asked not to use any medication which they would normally use for their premenstrual symptoms for at least two days before the premenstrual test days, or to take nutritional supplements during the study. The reproducibility of the occurrence of the premenstrual symptoms was assessed for one complete menstrual cycle by means of Freeman's daily diary of premenstrual symptoms (Freeman *et al.*, 1996). The sum of the 17 symptoms increased in the premenstrual period by 48% compared to the postmenstrual period, with increases of 44% on the mood subscale, 30% on the behavioral subscale, 69% on the pain subscale and 76 % on the physical symptoms subscale. The study was approved by the local hospital ethics committee. All subjects gave a written informed consent prior to participation.

Study design and method
On the two premenstrual days (between day 22 and 28 of the cycle) the participants received a controlled meal with either a chocolate drink containing the whey protein rich in α-

lactalbumin (treatment protein) or casein (placebo protein), according to a placebo-controlled, double-blind, cross-over design. Treatment order was counterbalanced. To assess cycle related changes in mood and appetite, participants were also tested in the post-menstrual phase of their cycle (between day 4 and 8). Premenstrual mood and appetite measures (defined as the average of the baseline assessments of the two premenstrual days) were compared to post-menstrual mood and appetite measures. Eight participants underwent the two premenstrual test days within two separate cycles (pre-post-pre), while seven participants were tested on two premenstrual test days in the same cycle with at least two days in between (pre-post order), of which three were in pre-pre-post order and four were in post-pre-pre order. This means that there were 14 premenstrual test days before and 16 premenstrual test assessments after the 15 postmenstrual days of the total of 45 test days, and indicates that the order of testdays was sufficiently balanced over the menstrual cycle.

Table I. Compositions and amino acid profiles of the chocolate drinks used in the α-lact[1] and casein diets.

	α-lact diet	Casein diet
Composition (g)		
α-Lactalbumin-enriched whey protein	20	0
Sodium caseinate	0	15.5
Cocoa	3.5	3.5
Granulated sugar	10	10
Water	200	200
Amino acid profile (g/kg)		
Isoleucine	27.61	31.80
Leucine	47.56	59.31
Phenylalanine	20.80	32.24
Tyrosine	16.82	33.13
Valine	29.52	44.09
Tryptophan	12.32	9.51
Trp:LNAA[2]	8.7	4.7

[1]α-lact diet, diet containing α-lactalbumin-enriched whey protein.
[2]Trp:LNAA, the ratio of tryptophan to the sum of the other large neutral amino acids.

Table II. Food components of the Standard diet and its energy composition.

Standard Diet	*Amount Used (g)*
Bread	70
Butter	30
Strawberry Jam	70
Red Grape Juice	500
Malt Bread	30
Mars bar	42
Chocolate Drink (α-lactalbumin or casein)	467
Percentage of Energy total diet including α-lactalbumin or casein chocolate drinks(%)	
Protein	11.7
Fat	24.8
Carbohydrates	63.5

Meals

The meals that were served on the two test days were isoenergetic containing 6133 kilojoules with 63.5 % of energy as carbohydrates, 11.7 % of energy as protein and 24.8% of energy as fat (see table II). The two diets were similar except for the composition of the chocolate drink in which the protein sources differed. The treatment chocolate drink con-

tained 20 grams of a whey protein concentrate rich in α-lactalbumin (Borculo Domo Ingredients, Borculo; The Netherlands), whereas the placebo chocolate drink contained 15.5 grams of the tryptophan-poor protein sodium caseinate (DMV International, Veghel; The Netherlands). Both chocolate drinks were identical to the drinks used in previous studies (Markus *et al.*, 2000b; Markus *et al.*, 2002) and were prepared by mixing chocolate powders, which were labeled with subject and test day number (1 or 2), with 200 ml water (see Table I for the amino acid profile of the chocolate drinks). The active dietary manipulation was served as breakfast, snack and lunch. The breakfast and lunch consisted of bread with butter and jam, malt bread (Soreen) with butter, black grape juice and the chocolate drink, and the snack was a chocolate bar (Mars) with grape juice.

Biochemical assessments

Blood samples were collected in 4 ml lithium heparin tubes by means of venapuncture and immediately placed on ice. The blood samples were centrifuged for 5 minutes at 4000 rpm at 4 ^{o}C within 30 minutes after collection. Subsequently 100 μl plasma was deproteinized by vortexing it with 6 mg sulfosalicyclic acid (SSA) (van Eijk *et al.*, 1994). The deproteinized plasma was frozen in dry ice and stored at –70 ^{o}C until quantative amino acid analysis by high-performance liquid chromatography (van Eijk *et al.*, 1993). To determine the plasma TRP/ΣLNAA ratio, plasma tryptophan was divided by the sum of the five other large neutral amino acids tyrosine, phenylalanine, leucine, isoleucine and valine,

Hourly Mood and appetite VAS ratings

Motivation to eat (hunger, fullness, thirst, desire to eat, prospective consumption, and nausea) and mood (anxiety, alertness, contentedness, irritability and enthusiasm) were rated several times during the test days using the Electronic Appetite Rating System (EARS), which is a validated computerised method to measure ratings of subjective state and appetite (Delargy *et al.*, 1996). Participants were asked to rate their subjective state on visual analogue scales (VAS-scale) using the EARS several times during the test sessions and at hourly intervals on a Psion palmtop computer after leaving the laboratory. Each question was framed in the same manner, for example: ‘How ‘hungry’ do you feel now?’. Responses were rated on a 100 mm line with anchors ‘not at all hungry’ (0) and ‘extremely hungry’ (100) placed at opposite ends. The participant moved the cursor to the left or the right and confirmed the rating, before the next question appeared on the screen. The ratings in the laboratory were made at t_0 (before and after the baseline test session), at t_4 and t_5 (before and after the afternoon test session), and hourly after leaving the laboratory until bedtime. For these hourly ratings the mean of two successive hourly sessions was calculated (t_6 and t_7, t_8 and t_9, t_{10} and t_{11}, and t_{12} and t_{13} respectively).

Mood VAS and PANAS

The Mood VAS was only assessed during the test sessions on the three test days, consisted of questions of the form: ‘How ‘angry’ do you feel now?’. The questions were presented in the following order: angry (-), calm (+), cheerful (+), clearheaded (+), confident (+), dejected (-), drowsy (-), energetic (+), friendly (+), jittery (-), lively (+), muddled (-), placid (+), tense (-), tired (-) and uncertain (-). The mean of the positive (+) and negative (-) VAS scales were calculated and used for statistical analysis. The Positive and Negative Affect Scale (PANAS) (Watson *et al.*, 1988) has been used to measure mood changes during or between the test days (Watson *et al.*, 1988). The participants were instructed to read each item and rate the

positive and negative emotions on a 1 to 5 scale (very slightly/not at all, a little, moderately, quite a bit, or extremely).

Food diaries
The participants completed food intake diaries on the day before each pre- or postmenstrual test day and on each of the three test days. 6 days food intake was recorded in all. Detailed information on how to record quantity and type of food and drinks consumed using common household measures were given inside the cover of the diary. Each day was divided into sections: before breakfast; breakfast; morning snack; lunch; afternoon snack; dinner and evening snack. At the end of each day the participants recorded their activity level, and indicated any events or feelings for that day. The food diaries were analysed with the dietary analysis program Diet5. If specific foods were not in the Diet5 database nutritional information was acquired from the product's label or from nutritional sources of food composition, e.g. website, manufacturer, or a book with information about the composition of foods (McCance and Widdowson, 1991). Diet5 was used to ascertain total energy intake (in kilocalories) and fat, carbohydrate and protein content (in percentages) from the food consumed (excluding the study diet during the test days) on treatment day 1 and 2, placebo day 1 and 2, and postmenstrual day 1 and 2.

Procedure
All participants underwent a complete training session prior to the test days. The participants were instructed not to drink alcohol on the day prior to the experiment, not to eat or drink (except water) after 10 pm that evening, and to arrive at the laboratory well-rested. In case of doubt about the regularity of the cycle, ovulation detection kits were used, to make sure that the cognitive assessments were in the premenstrual period. After their arrival at the laboratory the short list of mood and appetite visual analogue scale (VAS) ratings was completed two times (t_0) before and after the longer mood VAS and the PANAS questionnaire. Immediately after this the experimental meals were served on the premenstrual test days and an ad libitum breakfast on the postmenstrual test day. The short mood and appetite VAS ratings were repeated before and after the mood VAS and the PANAS questionnaire four hours (t_4) after start of the treatment. After the test sessions participants were asked to complete the short mood and VAS ratings every hour until bedtime. The participants also completed a daily symptom diary for a period of one month during their participation in the study (Freeman *et al.*, 1996).

Statistical analyses

All statistical analyses were performed using SPSS 10.0 for Windows. To assess treatment-related mood differences for the positive and negative scales of the PANAS and the positive and the negative mood VAS outcome variables for the two test days (placebo/treatment) a General Linear Model (GLM) analysis of variance for repeated measures was used. Within subjects factors were Meal (two levels: α-lactalbumin meal, casein meal) and Time (two levels: t_0, t_4). The food diaries were also analysed by using a GLM analysis with the within subjects factors Meal (two levels: α-lactalbumin meal, casein meal) and Time (two levels: day1, day2).
The hourly ratings of the six appetite and five mood VAS were separately analysed by means of a GLM analysis of variance for repeated measures, with treatment (two levels: α-

lactalbumin diet, casein diet) and time (six levels: mean two measures at t_0, mean t_4 and t_5, mean t_6 and t_7, mean t_8 and t_9, mean t_{10} and t_{11}, and mean t_{12} and t_{13}) as within subjects factors. Since the six questions of the hourly appetite and mood VAS scales were analysed separately post hoc comparisons using Bonferroni-Holme correction for multiple hypothesis testing were performed on significant effects (Holm, 1979).
Only the interaction of treatment x time of these analyses was considered for significance testing, since this is the treatment effect of importance. This treatment by time interaction effect is referred to as the effect of nutritional manipulation in the subsequent text.
The 'menstrual cycle' within subjects factor at baseline, was analysed separately as the difference between the average of the two premenstrual baseline assessments and the postmenstrual assessment (two levels: premenstrual, postmenstrual). This 'menstrual cycle' effect is referred to as effect of Cycle in the subsequent text. The average of the two premenstrual baseline assessments was used, because separate analyses showed that the two assessments did not differ.
To examine whether cycle phase order or treatment order influenced the results, these factors were added as between subjects factor in separate analyses. No effects of order were found, thus the results of the GLM without these between subjects factors are shown.

Results

Fifteen women completed the study. One participant withdrew after completing the casein (control) treatment because she suffered from pneumonia during her next premenstrual phase. Two volunteers did not continue after the training due to irregularity of their menstrual cycle.

Hourly Appetite and mood VAS ratings throughout the day:
Missing data for the appetite and mood VAS ratings throughout the day amounted to less than 10% of the ratings. The missing values were replaced with the series mean. The results are shown in Table IV. There was no effect of nutritional manipulation for any of the outcome appetite and mood VAS parameters. Significant menstrual cycle phase effects at baseline were present for the mood ratings anxious ($F_{2,13}$=10.97, p=0.005) and irritable ($F_{2,13}$=15.89, p=0.001). Participants felt more irritable and anxious during the premenstrual baseline test sessions than during the postmenstrual test session. With five mood ratings these effects remained significant after Bonferroni Holme correction. Menstrual cycle phase effects on the VAS scale ratings were also seen for the hourly ratings outside the laboratory. During the day after the postmenstrual test sessions participants felt more alert ($F_{3,12}$=10.12, p=0.001) than the day after the premenstrual test sessions. They also felt more nauseous ($F_{3,12}$=8.41, p=0.003) after the premenstrual test sessions than after the postmenstrual test session.

PANAS and mood VAS
The results of the positive and negative mood VAS scales and the positive and negative affective scales (PANAS) are shown in Table III.
No effect of nutritional manipulation was found for the positive mood VAS scales, the positive affect scales and the negative affect scales. However there was a significant effect of nutritional manipulation for the negative mood VAS scales ($F_{1,14}$=5.22, p=0.038). The negative mood VAS scales increased by 3.7 % after the casein meal and decreased by 1.2 % after the α-lactalbumin meal. No cycle effect was present for the positive affect scales of the

PANAS, whereas cycle effects were found for the positive mood VAS scales ($F_{1,14}$=6.17, p=0.026), the negative mood VAS scales ($F_{1,14}$=5.18, p=0.039) and the sum of the negative affect scales of the PANAS ($F_{1,14}$=9.52, p=0.008).

Table III. Means (Standard errors) of the mood VAS scales and the PANAS scales at baseline (t -1) and at 4 hours after the serving the meals. The postmenstrual cognitive assessment was only at baseline (t –1) (* p<0.05, ** p<0.01).

Laboratory Ratings	*Means and Standard errors at the different assessments*				*General Linear Models F-values*	
Mood VAS scales	*Treatment*	*t -1*	*t4*	*Postmenstrual*	*Effect of Meal*	*Effect of Cycle*
Mean Positive VAS scales	Alfalact	39.35 (2.33)	42.25 (2.60)	48.25 (2.35)	F1,14=3.73 p=0.074	F1,14=6.17* p=0.026
	Casein	45.56 (3.18)	42.94 (2.39)			
Mean Negative VAS scales	Alfalact	46.62 (2.12)	45.40 (2.76)	37.55 (2.66)	F1,14=5.22* p=0.038	F1,14=5.18* p=0.039
	Casein	42.06 (3.22)	45.76 (2.93)			
PANAS	*Treatment*	*t -1*	*t4*	*Postmenstrual*	*Effect of Meal*	*Effect of Cycle*
Sum Positive Affect Scales	Alfalact	16.87 (1.10)	16.13 (1.24)	19.33 (1.36)	F1,14=0.60 p=0.45	F1,14=3.19 p=0.096
	Casein	17.20 (1.09)	15.00 (1.06)			
Sum Negative Affect Scales	Alfalact	14.27 (1.26)	12.07 (1.38)	10.60 (0.51)	F1,14=0.001 p=0.97	F1,14=9.52** p=0.008
	Casein	14.33 (1.62)	12.20 (0.82)			

Overall, negative feelings increased after the casein meal and decreased after the α-lactalbumin meal, and there were more negative and less positive feelings during the baseline assessment of the premenstrual day than in the postmenstrual phase of the cycle, although the effect for the positive feelings was only demonstrated with the positive VAS scales and not with the positive affect scale from the PANAS.

Food diaries

The results of the analysis of the food diaries is shown in table V. No effect of nutritional manipulation or cycle was found for total kilocalories consumed or fat, carbohydrates and protein (as percentage of total energy) composition of the meal. Thus there was no fluctuation in the food intake selective consumption of specific nutrients during the premenstrual period . Moreover the α-lactalbumin meal did not influence food intake.

Biochemical variables

There was no main effect of cycle for the plasma tryptophan/ΣLNAA ratio. A significant effect of nutritional manipulation ($F_{2,10}$=8.84, p < 0.01) was found for the plasma tryptophan/ΣLNAA ratio. Differences in the plasma ratio of 38% at $t_{3½}$ ($F_{1,11}$=6.181, p<0.05) and of 70 % at t_5 ($F_{1,11}$=17.471, p<0.005) were observed after the α-lactalbumin meal compared to the casein meal (see figure I and table IV in chapter 6). Plasma Trp/ΣLNAA increased by 6% at $t_{3½}$ and 25% at t_5 compared to baseline after the α-lactalbumin meal, whereas plasma Trp/ΣLNAA ratios decreased by 22% at $t_{3½}$ and 25% at t_5 compared to baseline after the casein diet.

Table IV. Mean and standard errors and the GLM analyses of the Appetite VAS scales and the Mood VAS scales at baseline, after the meal intervention and throughout the pre- and postmenstrual test days until bedtime.

Daily ratings	*Means (and Standard errors) at the different assessments*							*F-values of the GLM*		
Appetite VAS scales	*Treatment*	*t1t2 baseline*	*t3t4*	*t5t6*	*t7t8*	*t9t10*	*t11t12*	*Effect of Meal*	*Cycle Effect Baseline*	*Cycle Effect Home*
Hungry	Alfalact	65.67 (5.13)	16.87 (2.20)	31.38 (3.90)	44.77 (5.61)	38.84 (5.21)	27.54 (4.84)	F5,10=1.53	F2,13=0.39	F3,12=1.05
	Casein	65.23 (3.99)	15.77 (3.12)	35.81 (3.83)	35.13 (3.41)	40.83 (4.83)	20.87 (2.74)	p=0.26	p=0.54	p=0.41
	Postmnst	63.37 (4.88)	39.57 (3.06)	36.48 (4.55)	41.42 (3.69)	42.08 (4.18)	33.98 (3.85)			
Full	Alfalact	20.47 (4.92)	68.47 (4.13)	51.38 (3.83)	46.81 (5.66)	49.15 (4.32)	57.12 (5.23)	F5,10=0.30	F2,13=3.36	F3,12=1.94
	Casein	20.03 (3.58)	71.50 (3.71)	54.23 (4.02)	54.02 (2.86)	48.81 (4.17)	60.92 (4.51)	p=0.90	p=0.09	p=0.18
	Postmnst	25.07 (4.73)	46.19 (3.16)	55.26 (4.45)	46.95 (3.20)	41.54 (4.28)	53.62 (2.92)			
Thirsty	Alfalact	59.8 (4.45)	53.83 (3.90)	59.5 (2.51)	56.66 (3.89)	50.01 (4.33)	49.62 (4.35)	F5,10=3.15	F2,13=2.65	F3,12=4.01
	Casein	66.93 (2.81)	60.8 (4.12)	48.02 (2.43)	47.75 (3.40)	52.02 (4.36)	48.82 (4.55)	p=0.06	p=0.13	p=0.034
	Postmnst	68 (4.06)	53.02 (3.14)	50.07 (4.23)	47.18 (3.78)	57.65 (2.90)	47.18 (4.26)			
Desire to eat	Alfalact	62.7 (5.61)	17.03 (2.31)	29.62 (3.58)	41.76 (5.78)	41.02 (5.90)	30.58 (4.53)	F5,10=2.18	F2,13=0.22	F3,12=0.74
	Casein	61.8 (4.48)	14.6 (3.14)	36.83 (4.15)	38.04 (2.99)	40.52 (5.00)	23.47 (3.21)	p=0.14	p=0.65	p=0.55
	Postmnst	60.57 (5.21)	35.36 (2.72)	35.27 (4.35)	42.77 (3.01)	42.59 (4.03)	35.45 (3.91)			
How much food	Alfalact	51.2 (4.74)	11 (2)	23.92 (2.74)	39.21 (6.56)	35.64 (5.46)	22.12 (3.80)	F5,10=1.30	F2,13=0.25	F3,12=2.32
	Casein	51.63 (5.91)	13.2 (3.25)	32.42 (4.47)	33.29 (3.45)	36.46 (4.52)	21.07 (3.21)	p=0.34	p=0.63	p=0.13
	Postmnst	53.03 (4.63)	34.71 (3.21)	30.77 (3.66)	36.60 (2.79)	39.74 (3.74)	32.07 (3.53)			
Nauseous	Alfalact	29.7 (6.17)	39.5 6.05 ()	32.81 (3.80)	34.38 (5.53)	25.26 (3.40)	16.19 (3.61)	F5,10=0.81	F2,13=5.70	F3,12=8.41
	Casein	31.5 (6.97)	36.23 (6.10)	28.29 (3.73)	21.60 (3.05)	20.50 (4.28)	18.90 (3.69)	p=0.57	p=0.032	p=0.003
	Postmnst	20.23 (4.08)	17.17 (3.29)	14.79 (3.11)	18.95 (3.17)	17.79 (3.61)	20.68 3.90 ()			
Mood VAS scales	*Treatment*	*t1t2 baseline*	*t3t4*	*t5t6*	*t7t8*	*t9t10*	*t11t12*	*Effect of Meal*	*Cycle Effect Baseline*	*Cycle Effect Home*
Anxious	Alfalact	46.47 (4.23)	33.97 (4.81)	39.54 (3.60)	39.52 (4.19)	33.69 (3.87)	35.81 (4.55)	F5,10=1.14	F2,13=10.97	F3,12=3.65
	Casein	42.87 (5.11)	35.37 (4.77)	37.27 (4.20)	45.58 (3.25)	39.45 (5.22)	32.89 (3.44)	p=0.40	p=0.005	p=0.04
	Postmnst	29.43 (4.26)	31.5 (4.15)	31.54 (3.84)	28.5 (4.36)	31.84 (5.25)	32.42 (5.01)			
Alert	Alfalact	37.97 (4.61)	42.5 (4.55)	48.31 (3.75)	52 (3.29)	48.80 (3.36)	46.92 (3.33)	F5,10=2.23	F2,13=4.09	F3,12=10.12
	Casein	42.8 (5.19)	40.13 (5.05)	48.04 (3.45)	51.17 (2.70)	50.83 (3.77)	44.49 (4.14)	p=0.13	p=0.63	p=0.001
	Postmnst	46.67 (5.06)	60.40 (4.28)	62.59 (4.50)	63.84 (2.54)	49.00 (4.08)	46.92 (4.54)			
Content	Alfalact	43.10 (4.44)	54.27 (2.99)	51.19 (2.83)	50.02 (2.30)	47.94 (2.96)	53.00 (2.92)	F5,10=2.06	F2,13=1.01	F3,12=1.43
	Casein	47.70 (4.56)	55.40 (3.36)	52.54 (2.70)	54.10 (1.77)	54.76 (3.10)	53.66 (2.53)	p=0.15	p=0.33	p=0.28
	Postmnst	49.03 (3.36)	57.36 (3.22)	58.13 (3.86)	62.53 (3.42)	57.38 (2.54)	60.61 (3.29)			
Irritable	Alfalact	54.30 (3.49)	38.10 (4.54)	44.31 (4.02)	49.74 (4.79)	45.98 (3.20)	44.42 (2.99)	F5,10=2.45	F2,13=15.89	F3,12=0.07
	Casein	49.27 (5.54)	39.90 (4.16)	47.15 (3.51)	44.69 (3.52)	44.46 (4.75)	39.81 (4.07)	p=0.11	p=0.001	p=0.98
	Postmnst	30.40 (3.90)	29.02 (4.06)	32.88 (4.11)	35.33 (4.40)	33.31 (4.70)	28.47 (3.69)			
Enthousiastic	Alfalact	37.13 (4.34)	41.83 (3.32)	40.00 (2.69)	43.76 (3.33)	42.52 (3.47)	42.81 (2.41)	F5,10=0.66	F2,13=1.05	F3,12=1.27
	Casein	42.30 (4.30)	41.97 (3.48)	43.27 (3.54)	43.63 (2.23)	42.58 (4.20)	42.73 (3.38)	p=0.67	p=0.32	p=0.33
	Postmnst	42.00 (4.19)	54.69 (3.67)	52.95 (4.02)	55.45 (1.78)	48.57 (4.37)	45.88 (3.52)			

Discussion

This study has identified impaired mood during the premenstrual phase in women with premenstrual symptoms compared to the postmenstrual phase, such that participants felt more irritable and less alert during the premenstrual phase. α-lactalbumin caused a significant increase in plasma TRP/ΣLNAA and, subsequently, improved the negative mood VAS ratings compared to the placebo treatment, casein. Energy intake and carbohydrate consumption was not increased in the premenstrual phase compared to the postmenstrual phase, and the α-lactalbumin meal did not influence the total energy intake and nutrition selection compared to the placebo. The participants felt more nauseous after the premenstrual assessments compared to the postmenstrual test assessment. This implies that the participants became slightly nauseous after consuming both premenstrual test meals.

Table V. Mean and standard errors and the GLM analyses of the food diaries on the day before and the test assessment days itself .

	Means and Standard errors		*F- and p-values of the GLM*	
	Pre testday (SE)	*Testday (SE)*	*Effect of Meal*	*Cycle effect*
Fat Alfalact (%)	35.43 (2.09)	35.60 (3.87)	F1,14=0.02 p=0.89	F1,14=0.09 p=0.77
Fat Casein (%)	38.51 (2.05)	37.78 (3.62)		
Fat Post (%)	36.26 (2.10)	33.85 (3.21)		
CH Alfalact (%)	47.25 (1.78)	46.72 (1.53)	F1,14=0.46 p=0.51	F1,14=0.02 p=0.89
CH Casein (%)	45.45 (2.21)	40.84 (3.47)		
CH Post (%)	47.131 (2.13)	46.30 (2.09)		
Protein Alfalact (%)	16.82 (1.20)	16.80 (2.26)	F1,14=0.69 p=0.42	F1,14=0.05 p=0.95
Protein Casein (%)	15.09 (0.88)	18.05 (1.83)		
Protein Post (%)	16.03 (1.17)	16.54 (1.51)		
Energy Alfalact (KC)	1796.20 (94.99)	1020.73 (134.51)	F1,14=2.15 p=0.17	F1,14=0.043 p=0.84
Energy Casein (KC)	1862.87 (124.34)	1552.33 (109.55)		
Energy Post (KC)	1865.00 (119.11)	826.13 (81.99)		

In a recent review, Dye and Blundell (Dye and Blundell, 1997) concluded that premenstrual energy intake was significantly higher than postmenstrual (i.e. follicular phase), in 25 of the 37 groups of women studied. We did not find the cycle differences in energy intake. One reason for this could be for the possible inclusion of women with dietary restraint, who show the tendency to restrict food intake consciously in order to maintain body weight or to promote weight loss, which can override the biological cyclical pattern of food intake (Schweiger *et al.*, 1992; Dye and Blundell, 1997). However, active dieters were excluded from the study. Interestingly, Schweiger and colleagues (Schweiger *et al.*, 1992) concluded that high cognitive restraint in everyday eating behavior may be a risk factor for the development of menstrual disturbance in young women. Cycle changes in carbohydrate consumption or other macronutrients were also not shown, although Brzezinski and colleagues have found increases in carbohydrate intake in the premenstrual phase (Brzezinski *et al.*, 1990). The failure to find cycle phase differences in energy and nutrient intake, may be due to sample size, or limited measurement of food intake within each menstrual cycle phase (one pre-test and one test day in each phase), or the categorization of high fat sweet foods as CHP in Brzezinski's study. The lack of menstrual cycle phase differences in total energy and nutrient intake, may explain the absence of an effect of the nutritional manipulation α-lactalbumin.

We found differences across the menstrual cycle in negative affect using the PANAS, and the positive and negative mood VAS-scales. Participants scored higher on the negative mood scale, and lower on the positive mood scale during the premenstrual phase baseline test sessions compared to the postmenstrual test session. This is consistent with previous studies (Evans *et al.*, 1998; Man *et al.*, 1999). Man and colleagues (Man *et al.*, 1999) studied mood changes in 10 PMDD women and 10 controls. They found that the PMDD subjects were moderately to severely depressed in the luteal (premenstrual) phase, but in the normal range in the follicular (postmenstrual) phase on the Beck's Depression Inventory (BDI) self-rating scale. In our study, serotonergic manipulation via the α-lactalbumin meal improved the negative mood VAS ratings in the premenstrual phase. The several SSRI treatments have also been shown to improve mood ratings such as irritability (Dimmock *et al.*, 2000). It therefore seems that serotonergic function in women suffering from premenstrual symptoms can be modulated by the nutritional intervention, α-lactalbumin.

Future studies need to demonstrate whether α-lactalbumin supplementation is potent enough to enhance mood ratings in women suffering from more severe cyclical or serotonin-mediated disorders. Plasma serotonin levels show a cyclical pattern during the menstrual cycle (Tam *et al.*, 1985; Ashby *et al.*, 1988), but plasma tryptophan/ΣLNAA ratio differences have not been studied in relation to menstrual cycle phase. Thus the effects produced by the α-lactalbumin intervention are consistent with a central serotonergic dysfunction in our sample.

In conclusion, women suffering from premenstrual symptoms felt more irritable and less alert during the premenstrual phase compared to the postmenstrual phase. Furthermore, α-lactalbumin reduced premenstrual negative mood in women suffering from premenstrual symptoms, presumably by elevating central brain serotonin levels, whereas premenstrual mood and appetite ratings are not influenced. Further studies, utilizing subchronic dietary treatments, are necessary to replicate this effect, before recommendations can be made about the suitability of α-lactalbumin as an adjunct or alternative treatment for serotonin-mediated mood disruptions during the menstrual cycle, usually treated with SSRI's.

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8 Concluding Remarks

The aim of the research described in this thesis was to study the influence of nutritional factors on cognitive functioning in otherwise healthy people with relatively mild cognitive impairment. For this purpose, two different groups of subjects suffering from memory complaints substantiated by objective cognitive tests were investigated: elderly individuals suffering from age-associated memory impairment (AAMI) and women of reproductive age suffering from premenstrual symptoms. In this thesis the treatment of mild cognitive impairment with nutritional interventions have been studied in both populations, although the mechanisms causing their cognitive impairments are different. Cognitive impairment in elderly individuals can be caused by numerous functional and structural changes in the aging brain, ranging from cellular and biochemical changes to changes in behavior. Cell death and oxidative stress are among the biological hypotheses put forward to explain age-related cognitive decline, whereas psychological factors, such as depression and mild mood changes, accelerate age-related cognitive decline (den Hartog, 2002; Visser et al., 2000). Other psychosocial factors, such as education and mental work load (Bosma et al., 2003a; Bosma et al., 2003b), are negatively associated with age-related cognitive decline and hence can be considered as protective factors. From the perspective of the studies described in this thesis, impaired functioning of neuronal membranes, caused by changes in phospholipid composition, and cholinergic (Mega, 2000) or serotonergic dysfunctioning are potentially relevant mechanisms for the mild cognitive impairments associated with aging. Several studies have suggested that these mechanisms can be modified by nutritional factors (e.g. Fernstrom, 1977). Different mechanisms may be responsible for the cognitive dysfunction seen in women suffering from premenstrual symptoms. For example, mild cognitive changes during the menstrual cycle have been linked to premenstrual hormonal changes (Kumari and Corr, 1998; Maki et al., 2002), and changes in serotonergic activity (Halbreich and Tworek, 1993). The aim of the nutritional manipulation described in this thesis was to reverse or counteract these serotonergic changes.

These concluding remarks will focus on three topics derived from the experiments, which are extensively described in the previous chapters in this thesis. The questions asked in the introduction will also be dealt with in the following sections. The findings of the trial investigating the effect of phosphatidylserine (PS) on cognition in elderly individuals with mild cognitive impairment are discussed first, followed by a discussion of the possibilities to treat the cognitive changes of women suffering from premenstrual symptoms with nutritional treatment aimed at increasing serotonergic function. Finally, the general pitfalls and possibilities of nutritional intervention as a way to improve cognition are discussed.

Does PS affect cognitive dysfunction in aging?

Whether soy-PS (S-PS) affects cognitive function was investigated in the study described in Chapter 4. Results showed, after critical examination of the outcome measures, that S-PS did not improve the cognitive functioning of individuals with AAMI or the more severe AACD subgroup. This indicated that neither was there a predicted general effect, nor a more subtle treatment response associated positively to the severity of cognitive impairment at baseline. However, results showed that S-PS in a daily dose of 300 or 600 mg is safe to use, based on standard biochemical safety parameters, hematological safety parameters, blood pressure, and heart rate. Thus should S-PS later prove to have a beneficial effect when aimed on

other outcome measures or other populations, it can be used without concern about possible side effects.

The choice for the cognitive test battery used in the S-PS study was based on the impairing test performance on these tests during aging, which has been studied in the MAastricht Aging Studie (Jolles et al., 1995). Another important factor was that the tests have previously shown to be appropriate for measuring cognitive changes, including both impairments and improvements, after nutritional manipulations (Riedel et al., 1999; Schmitt et al., 2000; Sobczak et al., 2002). Thus why did other researchers find an improvement of cognitive functioning in particular subgroups after S-PS treatment? Crook and colleagues (Crook, 1998) showed an improvement in the participants with low baseline scores in a study without a double-blind design. These authors compared the results of a study with only S-PS with the results of an earlier study that demonstrated a positive effect of bovine cortex PS compared to placebo (Crook et al., 1991). This is probably not the most effective, or methodologically sound, way to show that S-PS affects cognitive performance. The controlled study described in this thesis demonstrated that S-PS does not influence cognitive functioning.

In future studies, attention should be paid to the fatty-acid content of PS. Bovine cortex extract PS (BC-PS), which is no longer available because of concerns regarding the possible transmission of prion disease from bovine brains, may well be effective in improving cognitive performance in humans (Crook et al., 1991), whereas S-PS is not, and this may be due to differences in the fatty-acid composition of the PS molecule such as sphingolipids and sphingomyelins. In animal studies (Blokland et al., 1999; Sakai et al., 1996), there was a large difference in fatty acid content of the S-PS and BC-PS formulas used, and even between the different S-PS formulas. This might be important for the efficacy of specific PS formulas because certain fatty acids ratios have been linked to cognitive functions (Yehuda et al., 1999).

Other aspects that need to be addressed in future studies concern the production of S-PS and the possibility that it undergoes enzymatic degradation during storage, due to enzymes which may have been left over in the capsules after the production process, and the extent to which PS is taken up through the gastrointestinal tract and the blood-brain barrier after its oral administration. Previous studies in rats showed that only 0.25% of an injected dose reached the brain after 20 minutes (Orlando et al., 1987). Finally, the possibility that BC-PS may not really be efficacious in humans as well, should also be remembered.

Thus it can be concluded from the results of this thesis that S-PS does not have a beneficial effect on the cognitive function of elderly individuals suffering from mild cognitive dysfunction. However, this elderly population is not the only group for which it is claimed that S-PS improves cognitive functioning. Interest in the possible cognition-enhancing effects of PS arose in the 1980s, when PS was promoted as one of the first neuroprotective agents to be active in humans. Several peer-reviewed studies showed that BC-PS had positive effects on the functioning of neuronal membranes in animals (Cohen and Muller, 1992; Furushiro et al., 1997; Vannucchi and Pepeu, 1987; Zanotti et al., 1986). This was followed by some studies demonstrating improved cognitive functioning in patients with Alzheimer's disease (Amaducci, 1988; Delwaide et al., 1986; Delwaide et al., 1989) or AAMI (Crook et al., 1991; Gindin et al., 1993). In fact, when critically reviewed, the evidence is not very strong. More-

over, concern about the possible transmission of prion disease led to the withdrawal of BC-PS from the market. Not long after this, S-PS, a new PS supplement derived from soy was developed (Kidd, 1995). The present findings with S-PS are of importance because the claims concerning the cognition-enhancing effects of PS are based on data for BC-PS, without any empirical data based on treatment with S-PS. So far, our study is the only published controlled study of the effect of S-PS on human cognition. However, retailers still sell S-PS, claiming that it enhances human cognition. A search on Internet using the words 'soy phosphatidylserine' and 'sell' revealed 126 hits, of which 14 of the first 20 websites actually sell the supplement, while 19 of those 20 claim the mentioned unproven memory improving effects. Claims are for instance: 'Recent clinical studies show PS helps almost all people overcome dementia'. It should be mentioned that S-PS is not the only so called 'smart drug' without proven efficacy which is widely sold over the counter (Rose, 2002). The efficacy of these smart drugs needs to be studied thoroughly in future studies.

Serotonergic manipulations in women with premenstrual symptoms

A relation between lowered serotonin levels and cognitive dysfunction has been found in several diseases, such as Alzheimer's disease (Kumar et al., 1995; Porter et al., 2003) and depression (Cleare, 1997; Garcia-Toro et al., 2003). Serotonergic functioning can be manipulated in healthy volunteers by means of nutritional manipulations, such as acute tryptophan depletion (ATD), which induces a transient reduction in central serotonin concentrations. ATD has been repeatedly shown to induce memory dysfunctioning (Klaassen et al., 2002; Riedel et al., 1999; Rubinsztein et al., 2001; Schmitt et al., 2000). Interestingly, the study described in chapter 6 of this thesis showed that α-lactalbumin, which increases central serotonin concentrations, specifically improved visual memory functioning in women suffering from premenstrual symptoms. The increase in the plasma tryptophan/large neutral amino acids (ΣLNAA) ratio by 70% 5 hours after consumption of the α-lactalbumin meal (see chapter 6) is as impressive as the 79% decrease in the plasma tryptophan/ΣLNAA ratio 5 hours after ATD (Schmitt et al., 2000). While ATD is an experimental means to induce low serotonin levels, premenstrual symptoms may constitute a natural model of transiently low levels of 5-HT. Symptoms should be comparable to those elicited by ATD. A difference, however, is that in the premenstrual syndrome (PMS), besides the 5-HT system, the GABA system is also affected, especially via the neuroactive progesterone metabolite allopregnanolone. The full spectrum of PMS symptoms can be mimicked by progestagens and progesterone together with estrogen (Backstrom et al., 2003). Selective serotonin re-uptake inhibitors (SSRIs) and substances that inhibit ovulation, such as gonadotrophin-releasing hormone (GnRH) agonists, have proven to be effective treatments for premenstrual symptoms. Indeed, SSRIs are currently advocated as the gold standard for treatment of PMS (Pearlstein, 2002). This underlines the significance of new serotonergic treatments.

Given that both SSRIs and α-lactalbumin improve 5-HT function, an α-lactalbumin-containing diet may be a good nutritional alternative for the treatment of the premenstrual low mood and memory deficits of women suffering from less severe premenstrual symptoms, since improvements of memory (see chapter 6) and mood (see chapter 7) were found after α-lactalbumin. The finding that memory impairment during PMS could be reversed by the α-lactalbumin diet appeared to be specific for memory and did not generalize to other cognitive functions. We looked in particular at executive functions such as planning (see chapter 6),

measured with a computerized version of the Tower of London test. We also looked at appetite and food intake (see chapter 7), which did not appear to change throughout the menstrual cycle and which were not influenced by the α-lactalbumin diet. Further placebo-controlled studies should compare the cognition-enhancing effects of SSRI treatment and the α-lactalbumin meal in women suffering from premenstrual dysphoric disorder (PMDD), PMS, and less severe premenstrual symptoms, to determine whether the α-lactalbumin meal is as efficacious as an SSRI on memory function. Similarly, the profile of cognitive effects of these treatments needs to be compared, since studies conducted with the experimental rigor as the ones described in this thesis are lacking for SSRI effects on PMS and PMDD. Furthermore, the studies investigating the effects of SSRI on PMS and PMDD often lacked sophisticated cognitive test protocols.

Nutritional interventions and cognition: pitfalls and possibilities

Based on the search of the literature, described in chapter 2 and 3 of this thesis it was concluded that age-associated cognitive decline could be treated by nutritional interventions. However, the observed complete lack of efficacy of S-PS is not the first case in which a promising therapeutic substance for age-related cognitive problems fails miserably when tested in a truly controlled experimental study. In the past, a systematic methodological review of clinical and experimental effect studies concluded that ginkgo biloba had cognition-enhancing effects in cerebral insufficiency (Kleijnen and Knipschild, 1992), whereas recently no effects were found in elderly individuals with mild-to-moderate dementia or in individuals with AAMI in a rigorously controlled study (van Dongen et al., 2000).
Antioxidant substances to maintain or enhance cognitive function in older age have been studied as well. Lower concentrations of vitamin B12 and folate and higher concentrations of homocysteine were associated with poorer spatial copying skills in a study of 70 elderly male subjects (Riggs et al., 1996). Researchers tended to focus only on vitamin B12 instead of folate, even though vitamin B12 and folate are both involved in the conversion of homocysteine to methionine (Hunt and Groff, 1990), which is indirectly involved in the maintenance of the myelin sheath. Thus while there is indeed evidence of a positive association between nutrients and cognitive performance in elderly individuals, the efficacy of nutritional supplements has not been convincingly demonstrated in human experimental studies. This again underlines that in many areas the step from one paradigm to another is often too large. Here, we remain with the observation that nutrition affects cognition has been widely demonstrated in vitro, in behavioral experiments in animals, and in epidemiological studies, but not often in clinical studies of humans. For this reason, we have previously advocated the use of ‘experimental model studies’, aimed specifically at demonstrating the mechanisms by which nutrients act in humans in vivo (Riedel et al., 2003).
In chapters 2 and 3 of this thesis, a review of epidemiological studies showed there to be a positive association between a good nutritional status, consumption of certain nutrients (such as vitamins, herbal extracts, wine, fatty acids), and cognitive performance in the elderly. The question remained whether suppletion of these nutrients also enhances the cognitive functioning of elderly individuals in an experimental design. The results of the S-PS study in this thesis suggests that the efficacy of a nutritional supplement might depend on its source and composition. For this reason, as long as the efficacy of specific nutritional supplements has not been proven in randomized placebo-controlled studies, elderly individuals

should be advised to have a healthy diet that includes the recommended daily intake of micronutrients.

However, nutrition is not the only factor, which may influence cognitive performance in elderly individuals. Many other factors (see e.g. van Boxtel et al., 1998) have been shown to influence cognitive functioning, including mild traumatic brain injury (Klein et al., 1996; Matser et al., 1999), age-related changes in the brain structure (Tisserand, 2003), depression (Burt et al., 1995; den Hartog, 2002), or change in health status (Houx, 1991). For instance, disease or loss of a partner can result in altered diets (Kalmijn, 2000). These factors can influence the effects of nutrition and thus the results of the study. Other confounding factors, such as education and social economic background, should also be taken into account in future studies.

Another aspect which should be considered when studying the therapeutic benefits of certain nutrients is their potential toxicity (Fernstrom, 2000). This thesis presented the first placebo-controlled study of the safety of S-PS, even though the supplement has already been sold widely as a cognition enhancer for the elderly. Several nutritional supplements available over-the-counter are used in amounts exceeding the daily recommended intake, and often the safety of these doses has not been investigated.

To find out by which mechanisms certain nutrients or nutritional supplements of the ‘memory diet’ enhance cognitive functions, more experimental models that mimic the age-related decline in specific biological functions, resulting in cognitive impairment, are needed. Since aging is, like depression, associated with decreased plasma serotonin levels in humans (Kumar et al., 1998) and in rats (Yeung and Friedman, 1991), the effects of ATD can be studied in elderly individuals suffering from memory problems. These individuals may be more vulnerable to the memory-impairing effects of ATD than healthy elderly or young adults, due to their serotonergic vulnerability. However, it should be kept in mind that serotonin is not the only neurotransmitter whose levels change with age. Dopamine levels, which change with age, can also be influenced by nutritional manipulations. Acute phenylalanine/tyrosine depletion (APTD) reduces dopamine function, lowers mood, and impairs memory function (Harmer et al., 2001; Leyton et al., 2000). By combining ATD and APTD in a within subjects design, it may be possible to find out which mechanisms are sensitive to manipulation by nutritional interventions. Furthermore, the synthesis of acetylcholine in the brain, like that of serotonin, depends on substrate availability (choline), (Fernstrom, 1977), and can thus be influenced by the dietary availability of choline (Nakamura et al., 2001; Zeisel, 2000).

Finally, the quality of the placebo, an important methodological issue, should be addressed in future studies. In trials with medicines this is rather obvious, but in nutritional studies placebo capsules are not always identical to the treatment capsules. The first study with a truly identical placebo for ginkgo biloba was reviewed in Chapter 3 (van Dongen et al., 2000). In other studies the specific smell of ginkgo is easily recognizable, by researcher or even participants, which makes it unlikely that a study design can be truly double-blind. Interestingly, ginkgo biloba did not improve cognitive functioning in the study with the truly identical placebo. Another example is the serotonergic manipulation using a carbohydrate-rich and protein-poor diet (CR/PP) (Markus et al., 1998; Wurtman and Wurtman, 1988). The placebo diet is carbohydrate poor and protein rich (CP/PR), which makes it very difficult to compose a diet which is similar in taste and appearance to the CR/PP diet. An alternative to this manner of manipulating central serotonin concentrations is to use α-lactalbumin, as used in the studies of this thesis and previously by Markus and colleagues (Markus et al., 2000; Markus et

al., 1999). The placebo concept is still a major issue in studies with nutritional supplements, whereas it is already dealt with in studies using medicines. All European clinical pharmaceutical studies fulfill the requirements of the International Conference of Harmonisation Good Clinical Practice (ICH GCP) Consolidated Guidance for conducting clinical studies, which includes the requirement that the placebo should be identical in appearance and taste. However, it is only recently that these guidelines have been applied more often to nutritional studies. The European Parliament is currently implementing regulations regarding claims made about specific nutrients. According to European Directive 2000/13/EC, producers and manufacturers are free to provide whatever additional information they wish, provided that it is accurate and does not mislead the consumer. This Directive further prohibits the attribution to any foodstuff of the property of preventing, treating, or curing a human disease, or reference to such properties. In 2002, the Food Supplement Directive 2002/46/EC was published, listing all vitamins and minerals which may be used in the manufacture of food supplements. More laws and legislation on this topic are expected.

Conclusion

In conclusion, nutritional interventions have become popular since animal studies showed promising results, as described in chapter 4, but it is not easy to find comparable effects in humans. For example, nutritional substances are often administered intravenously or intraperitoneally in animal studies, yet these methods are not applied in humans. Moreover, orally administered nutritional supplements may not pass the blood-brain barrier or not even the gastrointestinal tract in humans. The choice of study population is also important. The AAMI criteria or other criteria such as AACD or MCI are based on symptoms of cognitive aging. Future research should not only be based on symptoms but rather on known mechanisms of specific populations. Focusing on serotonergic manipulation in women with premenstrual symptoms is an example of such a study. Additionally, this model is consistent with the concept of serotonergic vulnerability, which links mood and memory deficits in those subjects vulnerable to dysregulation of 5-HT systems (Riedel et al., 2002). Postsynaptic serotonergic responsivity fluctuates not only in women with PMS (Halbreich and Tworek, 1993) but, plasma serotonin levels have also been demonstrated to fluctuate in healthy women during the menstrual cycle (Hindberg and Naesh, 1992). Thus women suffering from premenstrual symptoms could serve as a ‘sensitive model population’ to study new serotonergic compounds for other indications such as major depression as well.

In summary, the studies described in this thesis showed that it is possible to evaluate claims about the therapeutic effect of nutritional interventions on neurocognition and related domains, provided that studies have a robust methodological design and make use of specific test batteries. Further research is needed in this fascinating area between healthy and successful cognitive functioning, and borderline conditions affecting cognitive functioning, conditions which have a high prevalence in western societies.

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Summary

Mild cognitive impairment is a common problem among aging individuals, whose cognitive capabilities slowly decline over the years. However, such complaints are not restricted solely to older individuals. Many women of reproductive age experience fluctuations in their mood and cognitive function throughout the menstrual cycle. These fluctuations are especially pronounced in women who suffer from the Premenstrual Syndrome (PMS). There is increasing interest in the possible cognition-enhancing effects of nutritional supplements, as reflected by the increasing number of publications regarding this subject. Statements regarding the efficacy of many nutrients or indices of nutritional status are often based on the results of epidemiological studies on cognitive functioning, whereas the effect on cognitive performance may be small. The aim of the studies described in this thesis was to learn more of the mechanisms underlying the effects of specific nutritional interventions on cognitive performance. The first chapters provide a review of recent studies into the efficacy of nutrients on cognition. The subsequent chapters describe studies in which it was investigated whether the phospholipid Soy-Phosphatidylserine can influence cognitive functioning in elderly individuals, and whether a nutritional supplement, α-lactalbumin, which theoretically boosts serotonergic activity in the brain, can improve cognitive function and mood of women suffering from neurocognitive and mood problems associated with the premenstrual phase of the menstrual cycle.

Chapter 1 outlines the aims and the scientific questions underlying the research described in this thesis. It reviews existing knowledge with regard to the processes of cognitive impairment in aging individuals and provides the definitions of age-related cognitive decline and related conditions. Cognitive impairment related to the menstrual cycle is also discussed in this chapter. The concept that nutritional factors may play an important role in the treatment of both premenstrual cognitive symptoms and age-related mild cognitive impairment is then introduced. Furthermore it is outlined why and how the studies in this dissertation are operationalised into experimentally designed studies to test these hypotheses.

Chapter 2 provides an overview of the literature on the association between nutrients or indices of nutritional status and cognitive performance. β-Carotene and α-tocopherol seem to facilitate cognitive performance in aging individuals, probably by influencing the metabolism of the antioxidant vitamins A and E. Furthermore, recent studies have demonstrated that folate, rather than vitamin B12, is positively associated with cognitive functioning. A critical review of the literature showed that Ginkgo Biloba, which contains flavonoids, terpenoids, and organic acids that are putative antioxidants as well, is positively associated with cognitive functioning. Finally, a proper diet (high carbohydrate/protein ratio and intake of micronutrients and glucose) and the consumption of low doses of caffeine have been associated with better cognitive functioning. In general, there appears to be a limited amount of experimental research on nutrients and cognitive function in humans, particularly in elderly individuals, even though there are several uncontrolled studies claiming cognition-improving

effects of nutritional supplements. The effects of the nutritional supplements discussed in chapter 2 were modest but do not seem to be very different from those of medicinal or investigational cognition-enhancing or anti-dementia drugs.

Chapter 3 gives an update of new findings on nutrients and cognitive functioning published between 1998 and 2001, and highlights the importance of human experimental models of altered cognitive functioning. Unlike epidemiological studies, experimental models can be used to mimic associations between nutrients and cognition, by manipulating their presumed mechanisms of action, and can eventually explain the causal nature of found associations. Epidemiological studies have established associations between nutritional status, consumption of vitamins, wine, fatty acids, and cognitive decline. This does not mean, however, that the depletion of certain nutrients can be corrected by a change of diet or by using nutritional supplements. Controlled clinical trials are needed to study these effects. A recent randomized, placebo-controlled Ginkgo Biloba study highlights the importance of controlled clinical trials. This study of the efficacy of Ginkgo Biloba in elderly individuals suffering from mild-to-moderate dementia or age-associated memory impairment did not show to have a beneficial effect on cognition, in contrast with previous Ginkgo Biloba trials. This was the first trial of Ginkgo in which the placebo capsules were identical in smell as the treatment capsules. This finding emphasizes the importance of the quality of the placebo.

The phospholipid phosphatidylserine (PS) is a nutritional supplement which is sold over the counter and which is also often advertised on the Internet as improving cognitive functioning in healthy people who are exposed to daily stress, in athletes, or in people suffering from decreased neural function due to aging. *Chapter 4* describes a randomized, placebo-controlled trial into the effects of soybean-derived PS (S-PS) in elderly individuals suffering from mild cognitive impairment. Participants were 120 male and female subjects, aged 57 years and older, who fulfilled the criteria for age-associated memory impairment (AAMI). A subgroup fulfilled the criteria for age-associated cognitive decline. The participants were randomly assigned to three treatment groups after the screening assessment: placebo, 300 mg S-PS daily, or 600 mg S-PS daily. Cognitive efficacy assessments, consisting of tests of learning and memory, choice reaction time, planning and attention, were scheduled at baseline, after 6 and 12 weeks of treatment, and after a single-blind wash-out period of 3 weeks. The primary outcome measures were delayed recall and recognition of a previously learned word list. S-PS did not have any effect on the outcome measures and no significant interactions between treatment and 'severity of memory complaints' were found. The study showed that S-PS does not fulfill the claim that it improves cognitive functioning in elderly people suffering from memory complaints.

PS can be prepared from several sources. The human tolerability of 300 mg bovine cortex-derived PS (BC-PS) has been demonstrated in earlier studies, whereas the safety of the widely advertised supplement S-PS has not been investigated. *Chapter 5* describes the results of a randomized, placebo-controlled study in which the safety of two dosages of S-PS (300 mg and 600 mg) compared to placebo was evaluated in elderly individuals with memory complaints. Standard biochemical and hematological safety parameters, blood pressure, and heart rate were assessed in the same AAMI population as described in chapter 4. The safety assessments were carried out at baseline, and after 6 and 12 weeks of treatment. No significant differences were found in any of the outcome variables between the treatment groups,

leading to the conclusion that S-PS is a safe nutritional supplement for elderly people if taken up to a dosage of 200 mg three times daily.

Women suffering from the Premenstrual Syndrome (PMS) experience fluctuations in mood and behavior during the menstrual cycle. The diminished mood and cognitive functioning, which occur 7-10 days before menses, may be related to abnormalities in serotonergic functioning. *Chapter 6* reports the results of a double-blind, placebo-controlled, cross-over study of the cognitive effects of a meal enriched with a high tryptophan protein concentrate rich in alpha-lactalbumin (α-lactalbumin), compared to a control meal with a low tryptophan protein (casein), given to women suffering from premenstrual symptoms. Fifteen women between 18 and 45 years, who reported experiencing premenstrual symptoms for more than 2 years, were tested during one postmenstrual and two premenstrual assessments. The cognitive test assessment consisted of verbal memory, visual memory, and planning tests. Further blood samples were taken to assess the ratio of plasma tryptophan to the other large neutral amino acids (TRP/ΣLNAA), giving an indication of the availability of tryptophan and serotonin in the brain. Visual pattern recognition memory was significantly impaired during the premenstrual phase relative to the postmenstrual phase. Furthermore, this visual memory impairment was ameliorated by the α-lactalbumin meal as compared to the casein meal during the premenstrual phase, and the plasma ratio of tryptophan to the other five large neutral amino acids increased significantly after the α-lactalbumin diet, which reflects an increased availability of tryptophan and serotonin in the brain. In conclusion, the α-lactalbumin meal alleviated the premenstrual visual memory deficits of women suffering from premenstrual symptoms, probably by enhancing central serotonin levels.

Low mood and increased appetite are also premenstrual symptoms which have been linked to serotonergic changes during the menstrual cycle. *Chapter 7* reports the results of a study into the effects of an α-lactalbumin meal compared to a control meal enriched with the protein casein on mood, appetite, and the TRP/ΣLNAA ratio, in 15 women (18-45 years) suffering from premenstrual symptoms. Visual Analogue Scales (VAS) of mood and appetite, the Positive And Negative Affect Scales (PANAS), and the TRP/ΣLNAA ratio, reflecting an increased availability of serotonin in the brain, were measured. To assess total daily energy intake and the nutrient content of their diet, participants also completed a food diary for 6 days. Mood and affective state declined during the premenstrual phase as compared to the postmenstrual phase. In particular, anxiousness and irritability increased, and alertness decreased during the premenstrual phase. Appetite and food intake did not fluctuate during the menstrual cycle. The α-lactalbumin meal significantly improved mood and, as described also in chapter 6, increased the plasma TRP/ΣLNAA ratio during the premenstrual phase compared to the casein meal. The α-lactalbumin meal did not influence appetite and food intake.

Finally, *Chapter 8* provides a general discussion and concluding remarks concerning the central question whether nutritional intervention can influence cognitive performance. The studies described in this thesis showed that it is possible to evaluate claims about the beneficial effect of nutritional interventions on cognitive performance and related domains, provided that a methodologically sound study design and sensitive, reliable and validated tests are used. The choice of study population is also an important factor for the design of studies. Future research should not only be based on symptoms but rather on known mechanisms or vulnerabilities of specific populations. This concept is supported in this dissertation by de-

monstrating a periodic vulnerability of the 5-HT system in women suffering from premenstrual symptoms. Further research is needed in this fascinating area between healthy and successful cognitive functioning, and borderline conditions affecting cognitive functioning, conditions which have a high prevalence in western societies.

Samenvatting

Lichte cognitieve klachten zijn een veel voorkomend fenomeen bij ouderen. Vaak gaan deze klachten dan ook gepaard met langzaam verslechterende cognitieve capaciteiten in de loop der jaren. Een geheel andere groep mensen bij wie enigermate verslechterde cognitieve capaciteiten worden aangetroffen zijn vrouwen die last hebben van het premenstruele syndroom (PMS). Deze klachten vertonen echter een cyclisch patroon. Wat beide groepen gemeen hebben is dat het om verslechterd cognitief functioneren van relatief milde aard gaat. De laatste jaren hebben onderzoekers steeds meer interesse in mogelijke cognitieverbeterende effecten van voedingssupplementen, wat heeft geleid tot meerdere publicaties op dit gebied. Beweringen over de effectiviteit van verschillende nutriënten zijn in de bestaande publicaties meestal gebaseerd op resultaten van epidemiologisch onderzoek waarin positieve relaties van indices van de voedingsstatus met cognitief functioneren zijn gevonden. Het primaire doel van dit proefschrift is het verschaffen van kennis over de mechanismen die aan de effectiviteit van de behandeling van lichte cognitieve klachten met voedingsinterventies ten grondslag liggen. De eerste hoofdstukken van het proefschrift geven een overzicht van de meest recente studies op het gebied van voeding en cognitie. Vervolgens worden onderzoeken beschreven waarin bestudeerd wordt of Soya-fosfatidylserine (S-PS; een glycerol molecuul met daaraan drie gekoppelde vetzuren dat zenuwcellen steviger zou maken waardoor ze weer sneller werken) het cognitief functioneren van ouderen kan verbeteren, en of cognitieve functies en stemming beïnvloed kunnen worden door α-lactalbumine (een eiwit met veel tryptofaan dat zenuwcellen die met stemming te maken hebben verbetert) in een onderzoekspopulatie van vrouwen die last hebben van verslechterde cognitieve functies en stemming gedurende de premenstruele fase van de menstruele cyclus.

Hoofdstuk 1 beschrijft het primaire doel en de wetenschappelijke vragen die gesteld zijn in dit proefschrift. Er wordt een overzicht gegeven alsmede een aantal definities rond het begrip milde cognitieve veroudering. Verminderde cognitieve prestaties gerelateerd aan bepaalde fases in de menstruele cyclus is eveneens een thema in dit hoofdstuk. Hierna volgt een uiteenzetting over de stelling dat voedingsfactoren een belangrijke rol kunnen spelen bij de behandeling van premenstruele cognitieve klachten en leeftijdsgerelateerde lichte cognitieve achteruitgang. Verder wordt weergegeven waarom en hoe de onderzoeken in dit proefschrift volgens een experimenteel ontwerp zijn uitgevoerd om de hypotheses te testen.

Hoofdstuk 2 verschaft een literatuuroverzicht van recente onderzoeken over de associatie tussen nutriënten of voedingsstatus en cognitief functioneren. Beta-caroteen en alfa-tocopherol lijken, waarschijnlijk door middel van stimulatie van de antioxidanten vitamine A en vitamine E respectievelijk, een positieve relatie te hebben met het cognitief functioneren van ouderen. Recente onderzoeken hebben ook aangetoond dat foliumzuur in plaats van vitamine B, zoals eerder werd aangenomen, positief gerelateerd is aan cognitief functioneren. Uit een kritisch literatuur overzicht werd geconcludeerd dat Ginkgo Biloba ook positief geassocieerd is met cognitieve prestaties, dat waarschijnlijk een gevolg is van de antioxidant

werking van haar componenten flavonoïden, terponoïden en organische zuren. Tot slot is een specifieke verhouding van koolhydraten en vetten, micronutriënten inname, glucose inname en consumptie van een lage dosis cafeïne, geassocieerd met betere cognitieve prestaties. Over het algemeen zijn er, met name in een ouderen populatie, weinig experimentele humane onderzoeken op het gebied van voeding en cognitie uitgevoerd. Echter, verschillende ongecontroleerde onderzoeken blijken cognitieverbeterende effecten van bepaalde voedingssupplementen te claimen. De grootte van de voedingssupplement-effecten op cognitie, die in hoofdstuk 2 worden besproken, zijn gering, maar niet kleiner dan effecten van cognitie verbeterende medicijnen die dementie zouden moeten tegengaan.

Hoofdstuk 3 geeft een overzicht van nieuwe bevindingen in de periode 1998 tot 2001 op het gebied van voeding en cognitie, en benadrukt het belang van humaan experimentele modellen van cognitieve functies. In tegenstelling tot epidemiologische studies, bootsen experimentele modellen associaties tussen voeding en cognitie na door middel van het manipuleren van de werkingsmechanismen, zodat inzicht in de mogelijke oorzaak-gevolg relatie zou kunnen worden verkregen. In epidemiologische studies zijn associaties gevonden tussen voedingsstatus, consumptie van bepaalde vitamines, wijn en vetzuren enerzijds en afname van cognitieve functies anderzijds. Dit betekent echter niet dat het tekort aan bepaalde nutrienten kan worden opgeheven door verhoogde inname via de maaltijd of door het slikken van voedingssupplementen. Placebo-gecontroleerde onderzoeken zijn van belang om deze effecten te bestuderen. Een recent gepubliceerd onderzoek in de gereviewde periode demonstreert het belang van gecontroleerde klinische onderzoeken. In een gerandomiseerde placebogecontroleerd onderzoek naar de effecten van Ginkgo Biloba in ouderen met milde tot geringe dementie of ouderdom gerelateerde cognitieve achteruitgang, verbeterde Ginkgo Biloba het cognitief functioneren niet, wat contrasteerde met eerdere Ginkgo Biloba onderzoeken. Let wel dat dit het eerste Ginkgo onderzoek was waarbij de placebo capsules ook wat betreft geur identiek waren aan de behandelingscapsules. De eerdere onderzoeksresultaten zouden dus wel eens allen gebaseerd kunnen zijn op een verwachtingseffect van onderzoeker en onderzochten vanwege doorbreking van de dubbel-blind code.

De fosfolipide Phosphatidylserine (PS) is een voedingssupplement dat zonder recept verkrijgbaar is en zelfs op internet verkocht met de claim dat het cognitieve prestaties verbetert bij gezonde mensen die aan stress worden blootgesteld, atleten of mensen die last hebben van verslechterd neuraal functioneren als gevolg van het ouder worden. *Hoofdstuk 4* beschrijft een gerandomiseerde placebo-gecontroleerd onderzoek naar de effecten van van sojabonen afgeleide PS (S-PS) bij ouderen die last hebben van lichte cognitieve klachten. De proefpersonen waren 120 mannen en vrouwen ouder dan 57 die voldeden aan de criteria van age-associated memory impairment (AAMI) oftewel lichte cognitieve klachten. Een subgroep voldeed ook aan de criteria voor age-associated cognitive decline (AACD) met ergere geheugenklachten. De proefpersonen werden na het screeningsonderzoek willekeurig toegewezen aan één van de drie behandelingsgroepen: placebo, een dagelijkse inname van 300 mg S-PS of een dagelijkse inname van 600 mg S-PS. De cognitieve uitkomstmaten waren accuraatheid en snelheid van prestatie op tests die oa maten: leren en geheugen, keuze reactietijd, planning en aandacht. De tests werden voor de behandeling, na 6 en 12 weken behandeling, en drie weken na de behandeling afgenomen. De belangrijkste uitkomstmaten waren vertraagde herinnering en herkenning van een eerder geleerde woordenlijst. S-PS had geen enkel effect op deze uitkomstmaten en er waren geen verbanden tussen de be-

handeling en de mate van geheugenklachten. In dit onderzoek is aangetoond dat S-PS niet voldoet aan de claim dat het het cognitief functioneren van ouderen met geheugenklachten kan verbeteren.

PS kan worden geproduceerd uit verschillende bronnen. De afwezigheid van bijwerkingen van een dagelijkse inname van 300 mg Bovine Cortex of rundercortex (BC-PS) was al eerder in verschillende onderzoeken aangetoond maar dit product is van de markt gehaald ivm. haar associatie met BSE. De veiligheid van het nieuwe supplement S-PS was echter nog niet eerder bestudeerd. In *hoofdstuk 5* zijn de resultaten van een gerandomiseerde placebo-gecontroleerd onderzoek naar de veiligheid van twee verschillende doseringen S-PS (300 mg en 600 mg) ten opzichte van placebo in een oudere populatie beschreven. Standaard biochemische en hematologische analyses van bloed, hoogte van bloeddruk en hartslagfrequentie werden gemeten in de onderzoekspopulatie welke reeds in hoofdstuk 4 is beschreven. De metingen werden uitgevoerd voor de behandeling, en na 6 en 12 weken behandeling. Er werden geen significante verschillen gevonden tussen de drie onderzoeksgroepen, wat leidde tot de conclusie dat het voor ouderen veilig is om het voedingssupplement S-PS te gebruiken tot een maximum inname van 200 mg drie keer daags.

Vrouwen die last hebben van het Premenstruele Syndroom (PMS) kunnen fluctuaties in stemming en gedrag vertonen tijdens verschillende fasen van de menstruele cyclus. Verslechterde stemming en cognitief functioneren rond 7-10 dagen voor de menstruatie kunnen gerelateerd zijn aan fluctuerende niveaus van hormonen en neurotransmitters zoals serotonine. In *hoofdstuk 6* worden de resultaten beschreven van een dubbel-blind, placebo-gecontroleerd, onderzoek naar de cognitieve effecten van een maaltijd met een toevoeging van het eiwit concentraat α-lactalbumine, dat veel tryptofaan bevat, vergeleken met een maaltijd met het eiwit concentraat caseïne, dat juist weinig tryptofaan bevat, in vrouwen die last hebben van premenstruele symptomen. 15 vrouwen tussen 18 en 45 jaar, die gedurende minimaal 2 jaar last hebben van premenstruele symptomen, werden één keer in de postmenstruele fase van hun cyclus getest en twee keer tijdens de premenstruele fase. De cognitieve testbatterij bestond uit de woordenleertaak, een plaatjesleertaak en een planningstaak. Eveneens werden bloedmonsters afgenomen om de ratio van plasma tryptofaan ten opzichte van de overige langeketen neutrale aminozuren (TRP/ΣLNAA), dat waarschijnlijk een indicatie geeft van de tryptofaan en serotonine concentraties in de hersenen. Onthouden van willekeurige visuele patronen verslechterde significant in de premenstruele fase vergeleken met de postmenstruele fase. Deze verslechtering van het visueel geheugen werd echter opgeheven door de α-lactalbumine maaltijd. Mogelijk was de geheugenverbetering een gevolg van de door de maaltijd veroorzaakte toename van de concentratie tryptofaan en serotonine in de hersenen. De conclusie was dat de α-lactalbumine maaltijd de verslechtering van het premenstrueel visueel geheugen opheft, bij vrouwen die last hebben van premenstruele klachten, waarschijnlijk via verhoging van centrale serotonine concentraties.

Verslechterde stemming en toename van eetlust zijn andere premenstruele symptomen die aan serotonerge veranderingen gedurende de menstruele cyclus gekoppeld kunnen worden. In *hoofdstuk 7* worden de effecten beschreven van een α-lactalbumine maaltijd in vergelijking met een controle maaltijd op stemming, eetlust en de TRP/ΣLNAA ratio in 15 vrouwen tussen 18 en 45 jaar die last hadden van premenstruele symptomen. Visueel analoge schalen (VAS) van stemming en eetlust, de ‘positive and negative affect scales’ (PANAS), en de

TRP/ΣLNAA ratio, die de beschikbaarheid van serotonine in de hersenen reflecteert, waren bepaald. Proefpersonen werden eveneens gevraagd om gedurende 6 dagen in een dagboek bij te houden welke voedingsmiddelen zij hadden genuttigd, om de dagelijkse energie inname en de nutriënten samenstelling van hun voeding te kunnen bepalen. Stemming en affectie verslechterden gedurende de meting in de premenstruele fase ten opzichte van de meting in de postmenstruele fase. Met name, angst en irritatie namen toe, terwijl de alertheid van de proefpersonen juist afnam tijdens de premenstruele fase. Eetlust en voedselinname fluctueerden niet tijdens de menstruele cyclus. De α-lactalbumine maaltijd induceerde een significante stemmingsverbetering en, zoals reeds beschreven in hoofdstuk 6, een toename van de TRP/ΣLNAA ratio gedurende de premenstruele fase, vergeleken met de caseïne maaltijd. De α-lactalbumine maaltijd had geen invloed op eetlust en voedingsinname.

Tenslotte zijn in *hoofdstuk 8* de algemene discussie en concluderende opmerkingen te vinden die betrekking hebben op de centrale vraag of voedingsinterventies cognitief functioneren kunnen beïnvloeden. De in dit proefschrift beschreven experimenten laten zien dat het mogelijk is om claims over de positieve effecten van voedingsinterventies op cognitieve prestaties en gerelateerde domeinen te onderzoeken, mits de experimenten met behulp van een methodologisch goed opgezet onderzoeksontwerp en gevoelige, betrouwbare en gevalideerde tests worden uitgevoerd. De keuze van de onderzoekspopulatie is eveneens een belangrijke factor voor het onderzoeksontwerp. Toekomstige onderzoeken moeten niet alleen gericht zijn op symptomen, maar eerder op bekende mechanismen of gevoeligheid van specifieke populaties. Een voorbeeld van dit concept in dit proefschrift was bijvoorbeeld de manier waarop de relatie tussen serotonine en premenstruele symptomen en cognitie werd onderzocht, namelijk door de effecten van manipulatie van serotonine te observeren. Bestaande claims over therapeutische effecten van voedingsinterventies op cognitieve prestaties, die zijn afgeleid van onderzoeken met een slecht onderzoeksontwerp, kunnen op deze manier beter onderzocht worden. Er is meer onderzoek nodig binnen dit interessante domein tussen gezond en succesvol ouder worden en condities met een hoge prevalentie in de westerse samenleving, waarbij het cognitief functioneren beïnvloed wordt.

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Curriculum Vitae

Brenda Jorissen werd op 10 oktober 1972 om tien over tien te Almelo geboren. Na het behalen van het Atheneum-β diploma aan het Christelijk Lyceum te Almelo en het Luzac College te Zwolle, ging zij Gezondheidswetenschappen studeren te Maastricht. In augustus 1997 heeft zij haar afstudeeronderzoek naar de invloed van tryptofaandepletie op cognitie afgerond bij de vakgroep Psychiatrie en Neuropsychologie (P&N) van de faculteit Geneeskunde, en behaalde hiermee haar diploma voor de afstudeerrichting Biologische Gezondheidkunde. Na een indrukwekkende reis naar Mexico, Guatemala en Belize kon zij als onderzoeksassistent beginnen bij de P&N onderzoeksgroep Experimentele Psychofarmacologie waar zij als stagiaire reeds werkzaam was. Het onderzoeksassistentschap werd na een paar maanden omgezet in het AIO-schap resulterend in dit proefschrift. Sinds 1 september 2002 is zij werkzaam als 'Clinical Quality Systems Specialist' bij het Medtronic Bakken Research Center te Maastricht.

List of Publications

Articles

- Jorissen BL, Schmitt JAJ, Dye L, Markus CR, Deutz NEP, Riedel WJ (in preparation) Dietary α-lactalbumin increases the ratio of plasma tryptophan to the other large neutral amino acids and ameliorates premenstrual memory consolidation deficit
- Jorissen BL, Dye L, Schmitt JAJ, Markus CR, Riedel WJ (in preparation) The effect of α-lactalbumin on mood and appetite in women suffering from premenstrual symptoms
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